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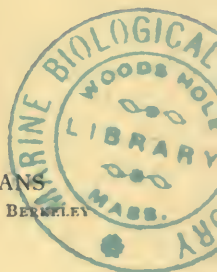
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THE PIGMENTARY, GROWTH AND ENDOCRINE DISTURBANCES INDUCED IN THE ANURAN TADPOLE BY THE EARLY ABLATION OF THE PARS BUCCALIS OF THE HYPOPHYSIS

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ANATOMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA

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CONTENTS

1. Introduction.....	5
The conditions of the experiment.....	7
2. Alterations in the pigmentary system.....	10
The chromatophores of the albinous larvae.....	14
The epidermal melanophores.....	14
The deep melanophores.....	18
The xantholeucophores.....	18
Epidermal transplants.....	23
The effect of various diets upon the pigment cells.....	27
The responses of the chromatophores to various physiological and pharmacological agents.....	30
3. Growth disturbances.....	41
The growth rate of the albino; the effect of the administration of various physiological substances upon this growth rate.....	44
The growth rate of thyroidectomized larvae.....	67
4. Modifications in the size and structure of the endocrine organs.....	68
The hypophysial components.....	69
The thyroid.....	83
The adrenal cortex and medulla.....	90
The epithelial bodies.....	97
The fat-organ.....	99
5. Discussion.....	100
6. Summary.....	103
Bibliography.....	109

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1. INTRODUCTION

Only within the brief period of four years has the favorable character of the early amphibian tadpole for analyzing the functional nature and the reactions of the members of the endocrine system been recognized. This may indeed seem strange, since the value of this material, to which attention was called by Born and which has been used so advantageously by Harrison, in the solution of those problems requiring experimental procedure upon the early embryo has long been appreciated. The early amphibian tadpole is peculiarly useful in experimental biological investigations, since in its earliest stages it is available for operation and has the inherent capacity to survive the most severe mutilations. These characteristics make a special appeal in studies upon the functional interrelationships obtaining in the endocrine system, for derangements in this system can be induced by the ablation of certain of its members in their early embryonal and non-functional stages by a simple operation, in itself not harmful. The early removal of a gland will afford then knowledge not only of the essentiality of this gland per se, but also concerning the dependence of the other endocrine organs upon this gland for their full development. This interdependence may be productive of even greater structural changes in the other glands than a later operation would produce, because of the lability inherent in embryonal structures. Further, the utilization of embryonal material would appear

¹ Aided by a grant from the Research Board of the University of California.

to be necessary in studies upon hypophysial extirpation if the prolonged survival of the animals is desired, since the complete extirpation of the epithelial hypophysis² in the adult invariably proves fatal within a brief period. And it would appear that only by the prolonged survival of the animal can the maximal alterations in the other members of this correlative system fully express themselves, as is shown by the structural picture presented by the endocrine organs of the 'hypophysectomized' tadpole. These organs exhibit structural changes exceeding in magnitude those obtaining in the hypophysectomized mammal. Indeed, it is possible to look upon these structural alterations as the expression of a restored functional balance in the endocrine system which has permitted the survival of the animal. One further advantage is bestowed by the use of embryonic material, for we may discover a more general influence of the endocrine organ in question on the manner or rate of development of all of the tissues and organs of the body—namely, on growth—and, as we shall see further on, growth effects are among the most prominent ones manifested by these disturbances.

In 1912 Gudernatsch reported that frog larvae could be metamorphosed by thyroid feeding. This remarkable result stimulated Adler in the same year to utilize the tadpole for the ablation of members of the endocrine system. By what now appears to be a crude method, Adler, in 1912, burned out the pituitary from half-grown frog larvae. Although the failures were numerous, the death rate high, and in the surviving successful cases the injury to the neighboring structures severe, nevertheless, Adler was able to show that the destruction of the pituitary impaired the thyroid gland and prevented metamorphosis. Subsequent and far more successful ablations of the hypophysis have amply confirmed Adler's findings.

In 1914 the author attempted to remove the epithelial anlage of the hypophysis in the early larvae of the newt—*D. torosus*. As had been the experience of other investigators, this urodele material proved unfavorable for early operation. The follow-

² That portion of the hypophysis arising from the oral ectoderm.

ing season unsuccessful attempts to transport living frog eggs from the Middle West were made. Finally favorable material was secured from the rather scanty local anuran fauna by collecting the adult pairs of the frog, *Rana boylei*, at the time of laying. The larvae of this form proved ideal, wounds healed rapidly, feeding was vigorous, and growth uniform.

An astonishing result of this early removal of the epithelial hypophysis was now revealed. Characteristic, silvery-colored larvae—albinos—in great contrast to their darkly pigmented normal mates, resulted. Similar results were obtained simultaneously by B. M. Allen. In a series of papers the writer, B. M. Allen, E. R. and M. M. Hoskins, and W. C. Atwell have further confirmed and sought to analyze this discovery. Allen has subsequently studied in more detail the changes induced by an equally early ablation of the thyroid gland. No one, it would appear, has endeavored to confine his attention to the altered anatomy and physiology displayed by these pituitary-free 'albinos.' The present monograph aims to do this. While brief statements of some of its material have appeared from time to time, there has been collected here for the first time as complete an account as is now possible of the pigmentary upset and other correlated bodily changes, especially endocrine alterations called forth by this procedure.

The conditions of the experiment

All amphibian material is not equally favorable for work of this nature. In order to assure prompt healing, the yolk must be moderate in amount and of a cohesive nature. Thus operative work upon the larvae of the common newt, *D. torosus*, is precluded, due to its large, abundant, and non-cohesive yolk granules, which extrude for hours through the wound, even final healing being prevented. The unfavorable character of the *Amblystoma punctatum* for work of this nature has previously been commented upon by Harrison. For studies on growth it is further essential that the animals be vigorous feeders and develop at a rather uniform rate. This desideratum would

apparently rule out one of the common California frogs, *R. aurora dratonyii*, as well as the newt. The frog, *Rana boylei* Baird, and to a limited extent the toad, *Bufo boreas*, were utilized in this work and have proved very satisfactory, since they recover quickly from the operation, are vigorous feeders, and exhibit a rather uniform rate of growth.

The anlage of the epithelial hypophysis can be more easily ablated than any of the other glands of internal secretion save the thyroid. As is well known, this ectodermal invagination, lying between the brain and the pharynx, is connected with the surface epithelium in very young frog larvae,³ and is consequently readily accessible (fig. 12). If a transverse cut with finely ground needles be made through the surface ectoderm between the forebrain protuberance and the pharynx⁴ (figs. 11, 12) and these two structures gently separated, the hypophysial ingrowth is readily distinguished lying on the ventral surface of the brain, from which it can be separated without serious injury to the latter. The stage selected for the operation should neither be too young, in which case the structures are undifferentiated and serious injury may be done to the mouth, nor too old, in which case the hypophysis will have migrated to its deeper position, thus making its removal extremely difficult. Larvae of $3\frac{1}{2}$ to 4 mm. in length, at which time the tail bud is well formed and the nasal placodes distinct, appear to be in the most favorable stage for epithelial hypophysectomy (fig. 11). Since reflex movement has not yet appeared, no anaesthetic is necessary.

It is essential to furnish a continuously renewed supply of well-aerated water to the larvae. In this locality the added necessity of 'sterilizing' the water by heat at 60°C. for at least an hour, followed by cooling and reaeration, has been forced upon us (Smith, '18), because of the pathogenic organisms which

³ Excellent descriptions of the development of this structure in Amphibia have been given by Orr ('89), Corning ('99), Kingsley and Thyng ('04), Atwell ('18), and others.

⁴ A depression at the point of invagination of the hypophysis plainly marks its position (figs. 11, 12, *Hyp. p.*). With later development this pit gradually disappears or merges into the stomodaeum.

apparently gain ingress through the city water supply. Experience during the past three seasons has abundantly justified this 'sterilization,' since infection has been inversely proportional to the extent to which this treated water was utilized. Such treatment of the water appears to impair in no way the normality of the animals raised therein.

With the daily feeding of special or check food substances, as has been done in this study, the necessity of removing the uneaten particles which would otherwise putrify has forced us to have special containers manufactured. Our needs were met by having vessels of two sizes cast. One size, made of a gray glazed earthenware, is 12 inches square by 6 inches deep; the other size, made of a gray porcelain, is 6 inches square by 4 inches deep. The water level in either type can be readily changed by an L-tube draining through a hole near the bottom of the container. It was found that from fifty to seventy-five specimens could be reared in each of the larger containers without overcrowding and from ten to twenty specimens in each of the smaller ones. The daily cleaning of such containers proved not to be an onerous task when done with a syphon cleaning tube fitted with an appropriately shaped inlet.

Various check and special food substances have been fed: liver, muscle,⁵ anterior lobe, posterior lobe of the hypophysis, adrenal cortex, adrenal medulla, as well as various extractives and residues of the anterior lobe of the hypophysis. As a check diet, finely ground fresh liver has proved more satisfactory than muscle, since the connective tissue of even finely ground muscle often unites fragments and thus prevents complete deglutition of partly swallowed series of food particles, resulting in the death of the animals. In all cases an abundant supply of boiled lettuce has been furnished. Although liver has proved satisfactory for the normal tadpole, yet by far the most adequate food substance for raising a vigorous, healthy albino is the fresh anterior lobe of the beef hypophysis. With this diet, which

⁵ Mendel and Osborne ('18) report that the proteins in both muscle and liver are adequate for the needs of nutrition in growth.

as will be shown replaces the growth substance lost by hypophysectomy, the albinos thrived and were much more healthy, vigorous, and enjoyed a greater longevity than with any other diet. This gland proved to be a very desirable food substance for the normal tadpole as well.

The alterations referable to hypophysectomy in frog or toad larvae express themselves in several ways. There early appears a pigmentary disturbance productive of a silvery tadpole—the albino. This system then exhibits profound structural and functional modifications. An early retardation in growth is apparent which progressively becomes more marked with development. The growth curves of these animals differ not only in their magnitude, but in their character when compared to the normal. And finally, most of the other members of the internal secretory system present a structural picture differing greatly from the normal.

It is thus convenient to present the matter under three headings:

Disturbances in the pigmentary system.

Alterations in the growth rate.

Alterations in the other glands of internal secretion.

2. ALTERATIONS IN THE PIGMENTARY SYSTEM

The striking color changes which many of the lower vertebrates exhibit under changed environmental conditions has long been known and early attracted attention to the mutual interplay of the various components of the chromatophore groups by which this chromatic ‘adaptability’ was effected. In addition to these environmental color changes, it has also been shown that pronounced changes in certain of the chromatophore groups can be experimentally induced: by the injection of adrenalin into the frog, Lieben ('06); by the immersion of the entire animal or portions of it in certain endocrine extracts, McCord and Allen ('17), Lowe ('17), Spaeth ('13 and '18, with fish scales); by experimental operative procedures, Lister ('58), Bimmerman

('78), Biedermann ('92), Hooker ('12), Redfield ('16); by electrical stimulation, Hermann ('86), Winkler ('10), Spaeth ('16, with fish scales); by pharmacological reagents, Lowe ('17); as well as by producing numerical and physiological modifications in the pigment cells by rearing the larvae in different backgrounds, Babák ('13).⁶ It is not surprising that a mechanism exhibiting such a functional lability would be modified by disturbances in the endocrine system; indeed, that this pigmentary mechanism is influenced by the internal secretory system has been suggested by Fuchs ('14) and Redfield ('16, '18). It was not known, however, until the reports of the writer and of B. M. Allen appeared in 1916 that the ablation of one of the endocrine glands would in itself profoundly and permanently modify the pigmentary system. It was then shown that the early ablation⁷ of the pars buccalis of the hypophysis induced pigmentary changes leading to the formation of a 'silvery' tadpole—the albino—the most striking pigmentary alteration as yet effected in the tadpole. In this pigmentary disturbance all components of the chromatophore system have been pictured as playing a significant rôle by various writers.

The epidermal melanophores have been shown to be diminished in number (Smith, Allen), in pigment content (Smith), and to display a persistent contraction (Allen), all of which has been substantiated in the recent article by Atwell and by the author. It has further been shown that the free melanin which lies near the outer border of the peripheral layer of epidermal cells suffers a pronounced diminution (Smith).

By this diminution in the melanin content of the epidermis, a modification which lends greater transparency to this epithelial covering, the iridescent quality of the subjacent chromatophore group—the xantholeucophores—which in the albino display a

⁶ The literature on the pigment cells is stupendous, only a glimpse of it being given here. A complete bibliography on this subject will be found in the comprehensive article of Fuchs in Wintersteins' *Handb. d. vergl. Physiol.*, Bd. 3, 1 Hälfte, 2 Teil.

⁷ An early operation appears to be essential for the production of a pigmentary effect, since Adler reports no striking pigmentary disturbance subsequent to hypophysectomy in the midlarval stages.

broad and persistent expansion, is permitted full effect. This striking and persistent expansion in the xantholeucophores or 'interference' cells appears to have heretofore escaped attention, yet in this phenomenon of albinism they play no secondary rôle, since to them is referable the silvery and iridescent quality of the albino.⁸

The contraction of the deep melanophores, first described by Allen and later confirmed by Atwell, who indeed refers the picture of albinism primarily to the altered physiological state of these cells, the author can corroborate for the young albinous tadpole, but repeated examinations have failed to reveal a definite contraction in the older albinous larvae of *R. boylei*. As will be subsequently pointed out, the physiological condition of these cells could make no significant contribution to the picture of albinism.

To anticipate, then, what will be more fully shown in this section, the essential pigmentary changes⁹ contributing to the picture of albinism in this form are three in number:

1. A diminution in the epidermal free pigment.

2. A diminution in the number and melanin content of the epidermal melanophores (because of their paucity in number and pigment content, the contraction of these cells plays no important rôle in the formation of this picture).

3. A great and persistent expansion in the xantholeucophores.

It is most essential in a study of this nature that the environmental condition be well known on account of the adaptability of the pigment system to such external factors. The standard environmental condition, which we believe closely approximates that obtaining in nature, was furnished by a diffuse light, a gray background and room temperature (18° to 25°C.). The ex-

⁸ I have recently called attention to the significant contribution which these cells make to the picture of albinism (Proc. Soc. Exp. Biol. and Med., 43-1418, 1919).

⁹ Although the pigmentary system of a hypophysectomized tadpole 12 mm. or even less in length shows variations from the normal, nevertheless, an animal two, or better, three or more times this size has these modifications more clearly differentiated. The descriptions, except when otherwise stated, were made upon animals raised upon an anterior-lobe diet and which have attained a size in excess of 45 mm. and were not less than 3½ months old.

treme variations from this 'indifferent' condition have been assumed to be a) a white background with direct sunlight; b) a black background and the absence of light, temperature in all cases being noted and regulated to suit the experimental desiderata.

The observations upon the pigment cells have been made both upon the well-illuminated living animal with the binocular microscope (a Zeiss instrument fitted with a water-immersion objective and no. 2 oculars; magnification, 42 diameters) and by examination of cutaneous whole mounts. By the first method the progressive changes in the pigment cells under altered environmental conditions can be noted, the observations in most cases of necessity being rapidly taken so as to exclude possible alterations resulting from the rather brilliant illumination necessary for binocular observation (as, for instance, in observing a dark-adapted animal). These observations have been checked and supplemented by skin whole mounts of the fixed animal, the fixation being so rapidly effected as to preclude any physiological alterations which might take place. Observations have been largely limited to the dorsal and neighboring lateral portions of the body, save in certain cases where the pigment cells lining the body cavity were noted in fixed specimens. Although observation of the deep melanophores of the dorsal region involves some difficulty more especially in the albino, in which they are largely masked by the expanded xantholeucophores, yet the observations herein reported were restricted to this region, since repeated observations have revealed the fact that the pigment cells exhibit regional variations both in the time and the magnitude of their response.

In this section of the paper there will be presented:

1. The anatomical and physiological characteristics of the pigment cells of the albino as compared with those of the unoperated tadpole and some remarks on the development of this condition.

2. The responsibility of the endocrine system for this pigment fault as shown by—

- a. Epidermal transplants.
- b. The effect of various pabula upon the pigment cells.
- c. The response of the chromatophores to various physiological and pharmacological agents.

The chromatophores of albinous larvae

As is well known, the melanophores of the tadpole are of two types. One type—the epidermal melanophore—is found in the epithelial covering of the body; the other type—the deep melanophore—lies in or around the deeper structures. These two types, then, of necessity, will be treated independently.

The epidermal melanophores. The epidermal melanophore, when in an expanded condition, presents an irregularly shaped body from which branched, slender processes radiate for relatively long distances (fig. 13). When in a greatly contracted condition, these processes are not evident and the cell body then is of a spherical or slightly irregular shape (figs. 19 and 21). All intermediate conditions between these two extremes can be seen with proper light and temperature conditions.

The melanophores of the epidermis are greatly reduced in number in the albino (figs. 13 and 14), many counts showing an average reduction of two-thirds from the normal number. Even in a greatly expanded condition these cells are usually separated by wide intervals, a condition which contrasts sharply with that obtaining in the normal animal where the expanded processes unite with, or even overlap, each other. Thirty-one counts from five specimens gave an average of 38 epidermal melanophores in a unit area of 0.36 of a sq.mm. Similar counts from normal animals reveal 119 to this unit area, thus exceeding by over three times the number present in the albino (table 1). It is emphasized, however, that the distribution of these cells in neither type of animal is uniform. Yet their number in the normal even in the areas where they are most thinly distributed exceeds very considerably their number in the albino where they are most thickly placed. Further, the actual melanin content of these cells is diminished in the albino (figs. 13 and 20). Their processes, slender and of light color, present a very differ-

ent appearance from that furnished by the coarse black processes of the normal melanophore.¹⁰

Not only do these superficial melanin-bearing cells exhibit differences of a quantitative nature in these two types of animals, but constant physiological dissimilarity is also encountered. It may be urged that the functional states of these cells in the normal usually exhibit such wide variation not only between two or more individuals, but also in the cells of a single individual, that the determination of a normal physiological condition is precluded. Certain it is that not only do we find differences between the condition of the individual cells of a specimen, but under identical environmental conditions these cells in one animal may be expanded while those of its mates are partly contracted.¹¹ Yet this variability is not so great as to preclude our defining the condition of these cells in a normal animal in an 'indifferent' environment as one of complete or nearly complete expansion (fig. 13).

With the albino, as with the normal, but to a lesser extent, the same variability in the superficial melanophores of various individuals is exhibited. The individual cells of any one specimen, however, usually exhibit a greater dissimilarity than in the normal animal. A very few of these cells are almost invariably broadly expanded, a few are half expanded, while the majority are completely contracted or but slightly expanded (fig. 14). It is apparent, then, that the usual physiological

¹⁰ The evidence on this point is quite clear; corroborative evidence has also recently been furnished by Atwell, although Allen is apparently not in agreement with these findings, for he says: "These observations and a careful study of the pigment cells convince me that there is no disappearance and bleaching of the pigment cells as asserted by Smith." It is worthy of mention here that the author has never mentioned a "disappearance and bleaching of the pigment cells," stating, "Counts of the epidermal melanophores—in the albinos and cheeks show that the number of these cells in the epidermis is reduced in the former" and—"the melanophores of the albino specimens contain fewer pigment granules than do those of the cheeks and thus have a distinctly lighter appearance." The process leading to this condition was not then discussed by the author.

¹¹ This variability has led certain investigators to confine their observations in various physiological experiments to the more uniformly reacting deep melanophores—for example, Laurens.

TABLE 1
Table showing the number of epidermal melanophores in a unit area of 0.36 of a sq.mm.

Individual specimen number	SPECIMEN				MELANOPHORES	
	Type	Length ¹ in mm.	Age, dated from operative stage (days)	Diet	Number of counts	Average number per unit area
9	Normal frog tadpole	17 (total length)	29	Liver	5	111
11	Normal frog tadpole	25 (total length)	39	Liver	6	122
17	Normal frog tadpole	43 -/-/6.0	101	Liver	4	92
19	Normal frog tadpole	43 -/-/9.0	101	Liver	4	144
31	Normal frog tadpole	40 -/-/10.	144	Liver	7	127 Average of 26 counts = 119
10	Albino frog tadpole	16.5 (total length)	29	Liver	4	53
12	Albino frog tadpole	23.5 (total length)	39	Liver	6	28
16	Albino frog tadpole	43 -/-/0.1	101	Liver	7	45

18	Albino frog tadpole	43 -/-/0.1	101	Liver	7	32
20	Albino frog tadpole	40 -/-/0.1	144	Liver	7	30 Average of 31 counts = 38
38	Albino frog tadpole	47 15/32/0.1	135	Posterior lobe	7	51.4
40	Albino frog tadpole	46 15/31/0.1	141	Posterior lobe	5	52
42	Albino frog tadpole	42 14/28/0.1	144	Posterior lobe	3	64
44	Albino frog tadpole	44 14/30/0.1	144	Posterior lobe	3	47 ^a
46	Albino frog tadpole	42 15/27/0.5	174	Posterior lobe	3	43 Average of 21 counts = 51
p 2	Partial albino frog tadpole	45 18/27/6.5	148	Liver	8	52
p 3	Partial albino frog tadpole	43.5 16.5/27/6	243	Liver	5	49
p 6.	Partial albino frog tadpole	42 15/27/10	282	Anterior lobe May 6-July 18; then liver	4	49 Average of 17 counts = 50

^a The length of the component parts of the tadpole is given in the following order: Total Body/Tail/legs /Hind/Fore /legs

condition obtaining in these cells in the albino is one of complete, or nearly complete, contraction.

The free pigment which forms a definite, though imperfect, layer in the normal animal is much diminished in amount and is irregularly distributed in the albinous larvae (figs. 13, 14). In the skin whole mounts of the albino, it indeed forms but a scanty and irregular sheet which stands in contrast to the very distinct layer present in the normal animal.

The deep melanophores. The number and melanin content of the deep melanophores apparently are not altered by the ablation of the hypophysis. This structural independence appears not to lend support to those investigators who assert that both groups of melanophores, epidermal and corial, arise from a common source.¹²

That the deep melanophores exhibit a contracted condition has been noted by Allen and Atwell, the latter, indeed, referring the phenomenon of albinism primarily to their contracted condition. Many examinations of larvae in excess of 45 mm. in length, made both on the living animal and after rapid fixation, convince the author beyond question that in the body and head regions these cells, whether occurring in the corium, in muscle sheaths, or in the fascia of the viscera and body cavities, exhibit nearly the same physiological state in the fully developed albino of *R. boylei* as in the normal (figs. 13, 14); in the young albino, however, certain of these cells appear to be in various stages of contraction (fig. 16).

The xantholeucophores. We have noted significant and constant changes in the superficial layer of the first group of pigment cells—the bearers of melanin. Changes no less significant in the phenomenon of albinism are encountered in the second group of chromatophores, the bearers of guanine and xanthine. Indeed, since the silvery and iridescent characteristics of many fishes are referable to these cells, it would seem to make them of paramount importance in the phenomenon of albinism. When viewed by transmitted light, these cells are of a grayish or brown-

¹² Ehrmann ('96 and earlier) refers the common origin of the melanophores to the mesoblast of the dorsal cephalic region of the body.

ish color. It is, however, with reflected light that their great beauty and their contribution to the color of the animal becomes apparent. For one constituent, the guanine, distributed in minute crystals reflects the light and lends to it the iridescent qualities; the other constituent, the xanthine, small in amount in the tadpole, contributes to this color effect.

Delicate in structure and apparently exhibiting no resistant cell membrane, these cells are prone to disintegrate, consequently appropriate and rapid fixation is essential for their preservation. This delicacy is well shown in the epidermal transplants where although but a few seconds may lapse before the graft 'takes,' yet these cells in any region where an intimate union between host and graft does not obtain, change their character and ultimately disintegrate. Or, indeed, even when not subject to mechanical disturbances, as following the natural death of their host, they are wont to lose quickly their cellular integrity, forming a diffuse layer of fine crystals, which exhibit a vigorous Brownian movement—a most striking picture with the polariscope. Not only is early and rapid fixation essential, but appropriate fixers must be employed. This is well shown if a specimen be dropped into formol. Such an animal after a few weeks, or even days, loses its silvery appearance and assumes a gray tone. Microscopic examination reveals the fact that the xantholeucophores are no longer visible.

Over the dorsal body areas the xantholeucophores form two well-defined layers, each layer being composed of discrete independent cells. The peripheral layer, lying just beneath the epidermal basement membrane, is usually separated by a considerable interval from the deeper layer which lies adjacent to but external to the corial melanophores. In those areas where the epidermis closely approximates the underlying firm body structures, these two layers of xantholeucophores, except under high magnification, may appear as a common layer. We thus see that the iridescent cells lie between the two types of melanophores, an arrangement the recognition of which is of no little moment in evaluating the rôle of the various groups of pigment cells in albinism.

Both layers of corial xantholeucophores are broadly expanded in the albino (figs. 14, 16, 18, 20 to 23, 55 to 58), an expansion which is singularly unamenable, more especially in the older albinos, to altered environmental conditions. To the broad expanse of the refractive cells the albino owes its metallic tone, as can be clearly shown by all procedures which contract these cells.

We have thus far confined our attention to two types of tadpoles, that type in which the tadpoles have suffered the entire loss of the buccal component of the pituitary, the albino, and that type whose members have suffered no operative interference, the normal. It is now desirable to consider a third type—those in which the epithelial hypophysis was incompletely ablated, the 'partial' albino (figs. 52 to 54). This type of tadpole usually suffers a serious pigmentary disturbance, its pigmentary system simulating that of the albino more than that of the normal. In fact, its almost typical albinism frequently makes this animal difficult and often impossible to distinguish during early development from its completely hypophysectomized brothers. Later in life it is readily distinguishable, since legs develop in the partially but not in the completely hypophysectomized tadpole. In the albinous type of partially hypophysectomized tadpole as in the typical albino the free pigment is greatly diminished in amount and the epidermal melanophores are scanty in number. That the epidermal melanophores are slightly greater in number than in the complete albino, however, is apparent from counts. It will be recalled that thirty-one counts from five albinos gave an average of thirty-eight epidermal melanophores to a unit area 0.36 of a sq.mm. Seventeen similar counts from three 'partial' albinos gave an average of fifty to this unit area (table 1). The xantholeucophores appear to be no way different from those of the typical albino. They exhibit a broad and persistent expansion. Although the pigment system of this animal closely resembles that of the albino, yet, in contrast to the albino, it does not suffer serious structural defects in any of its endocrine organs save one, the neural component of the pituitary. Exhibiting, then, characteristics of both the albino and the normal, this 'hybrid' tadpole has been

of especial value in freeing any of the other endocrine glands which have been studied from responsibility for the pigmentary disturbance, as will be later shown.

An examination of the pigmentary system of the albino has revealed that one group of chromatophores, the xantholeucophores, exhibits a great expansion; another group, the epidermal melanophores, is diminished both in number and pigment content and displays an unusual contraction. It is necessary, then, to examine the developmental stages leading to the production of the characteristic albino if we wish to determine whether this is a progressive phenomenon; whether from the beginning the formation of the epidermal melanophores, for example, is inhibited by the endocrine fault, or whether on the other hand they form only to partially disappear later. The three groups of pigment-bearing cells make their appearance in the following sequence: deep melanophores, xantholeucophores, epidermal melanophores. A period of several days intervenes between the appearance of each of these groups, and so they may well be treated independently in a developmental study.

The time of appearance of the deep melanophores apparently is identical in both the normal and hypophysectomized tadpole. In a 6 to 7 mm. stage for the normal, they have already made their appearance in the dorsal regions of the head and body. A few show the typical shape of the deep melanophore. A majority, however, have longer and more delicate processes than the typical corial melanophore. They either lie well separated from the epidermis or, especially where the epithelium closely approximates the underlying firm structures, may be in contact with the epithelial covering. From this stage they progressively increase in number and gradually become organized until they attain the typical arrangement as a corial sheet at a 10 to 12 mm. stage (fourteen to twenty days after the operative stage).

The xantholeucophores make their appearance in larvae of 10 to 11 mm. (fourteen to sixteen days after the operative stage), appearing concomitantly both in time and number in the normal and hypophysectomized larvae. Slightly more numerous, pos-

sibly, in the hypophysis-free specimens, this numerical difference is so slight as to be well within the limits of variability. From their earliest appearance they are expanded in the operated tadpole (fig. 16) in contrast to their punctate character in the normal (fig. 15). The early, almost imperceptible albinous appearance which denotes a successful hypophysis extirpation is due largely to the expanded condition of these cells, since in these early stages the free epidermal pigment in both albinous and normal larvae is identical and the epidermal melanophores have not as yet formed in either type of specimen. Scarcely perceptible at first, this whiteness of the hypophysectomized tadpoles progressively increases with the increase in the number of the xantholeucophores, and is further emphasized by the reorganization and diminution in the early embryonic pigment which takes place, as will be described later.

In the diminution in the epidermal melanin we find the first structural pigmentary alteration referable to hypophysectomy. Here we have to deal with the double expression of the melanin, that existing as free granules and that included in the chromatophores. In the earliest stages free pigment is found diffusely scattered throughout the two cell layers of the epidermis, although occurring more plentifully in the outer of these layers and in certain cells of this outer layer. At a 10 mm. stage it has become localized in the superficial layer of the epithelium, and by the time the larvae have reached approximately a 20 to 25 mm. stage it is found only in the peripheral zone of this cell layer.¹³ Identical in amount in the earlier larval stage of the operated and normal larvae (figs. 15, 16), it gradually becomes diminished in the albino (figs. 17, 18), a diminution which aids in permitting the iridescent qualities of the xantholeucophores to be fully expressed and which is shown by the rapid development of the albinous picture at this time (figs. 42 to 45).

The first typical epidermal melanophores make their appearance in about a 10 mm. normal (twenty-two days after the operative stage, fig. 15). At first scatteringly distributed and

¹³ The changes here described are nearly identical with those described by Maurer ('95).

light in color, these cells rapidly increase in number and melanin content; by a 15 mm. stage they have numerically and structurally attained the mature larval condition (fig. 17, table 1). Their appearance in the albino is more tardy. They do not appear as early and are always diminished in number and melanin content as shown by figures 16 and 18, which were drawn from albinos of the same age and size as the normals from which figures 15 and 17, respectively, were drawn. A partly contracted condition is also evident from their earliest appearance.

No evidence has accrued from these studies which suggests that the diminution in the number of epidermal melanophores suffered by the albino is affected by a migration of the epidermal melanophores into the corium as suggested by Allen. On the other hand, the evidence all points to the developmental and functional independence of the two systems of melanophores. The first melanophores to appear are those in the corium, and there is at no time any evidence that their number is augmented by a migratory process from the epidermis. The formation of the epidermal melanophores from the beginning is apparently partially inhibited in the albino, an inhibition which is expressed not only in the diminished number of cells, but in their melanin content as well.

One of the remarkable features in this pigmentary disturbance is its early appearance. Whether referable to some missing hormone of the anterior lobe or to other hormonal disturbances provoked by the operation, it appears clear that hormones are already produced by the small groups of embryonic cells constituting the endocrine glands at a time when the latter exhibit but little if any of the structural differentiations which characterize the adult internal secretory organs.

Epidermal transplants

We have detailed at some length the pigmentary disturbances which result from the early loss of the epithelial hypophysis in the tadpole. It is now of interest to inquire whether this functional upset is due directly to an alteration in the quality of

the fluids bathing these cells or, on the other hand, to alterations in their nervous mechanism.

Unimpeachable evidence has been secured on this point by reciprocal skin transplants. In a very considerable number of cases skin interchanges have been successfully effected between a normal tadpole and its albinous mate. *Invariably, and in a period not exceeding four hours for the xantholeucophores and a somewhat longer period for the epidermal melanophores,¹⁴ these cells of the transplant assume the state characteristic for the corresponding cells of the host.*

The technical manipulations involved in performing these skin exchanges even in larvae exceeding 40 mm. in length are not difficult. A narcosis sufficiently deep for operative work has not readily been effected at room temperature, although serving well for ordinary examinations in which no trauma was inflicted. Of the several anaesthetics employed, ethyl eurethane (Merk) in a strength of 0.5 per cent has proved the most efficacious, especially when used at a low temperature (1° to 3°). The lighter narcosis effective at this low temperature is of no little significance, since not only is recovery more certain, but also functional changes in the pigment cells inherent in a profound anaesthesia are avoided.

After a sufficiently deep insensibility had been produced, the specimens were placed closely together in depressions in the wax plate lining the dish. Incisions on the dorsal epidermis mapping out an area approximately 4×6 mm. were then made on the dorsum of each specimen with the aid of the binocular. Following this the skin was rapidly freed and the interchange effected, the grafts being gently pressed down and not infrequently tucked under the edge of the host's skin. These grafts quickly adhered, and in a short time no movement of the host could dislodge them.

¹⁴ This reaction of the epidermal melanophores of the albinous graft to the normal host—one of expansion—applies only to the cells originally belonging to the graft. Epidermal melanophores from the surrounding normally pigmented epithelium of the host quickly invade the albinous graft. These cells display a state of great contraction for at least a considerable interval.

There now was exhibited one of the most striking phenomena presented in this work. The heretofore broadly expanded xantholeucophores of the albinous graft gradually contracted under the influence of its normal host. In half an hour they were much reduced; in two hours they were punctate, even exceeding in minuteness under this new stimulus the diminutive size of the host's 'interference' cells (figs. 57 and 58). Likewise the epidermal melanophores of the albinous graft gradually expanded. This change from the altered physiological state of these cells in the albino to the state characteristic of the normal animal then persisted for the life of the host.

A change as striking was exhibited by the heretofore contracted xantholeucophores and expanded epidermal melanophores of the normal graft. In about the same interval the xantholeucophores of the normal graft became broadly expanded (figs. 57, 58), and after a somewhat longer interval the epidermal melanophores assumed a punctate condition. These cells, then, in this brief interval had changed from the functional state they had so long experienced to the opposite condition exhibited by them in the new host.

There is, however, the possibility that this remarkable transformation was due to the mechanical manipulation and not to the influence of the host. This possibility can definitely be excluded by the simple test involved in an autoplasmic skin graft. Identical incisions were made, the freed epidermis, however, instead of being transferred to a new host, was returned to the position from which it for a brief interval had been removed. In neither the normal nor albinous larvae did these cells suffer any permanent change, but remained in the same condition as the cells which had not been removed.

Definite evidence has thus been secured which shows that the altered state of these cells in the albino has been brought about by the action of some substance present in the tissue fluids and not by alterations in the nervous mechanism, since we can conceive of no reestablishment of nervous connection in such a brief interval. Further, it would seem probable that this substance acts directly upon the pigment cell itself and not upon

the severed nerve termination. This would appear to be the case, since if this atypical condition exhibited by the cells of the normal graft to the albinous host was due to the action of the tissue fluids upon the severed motor connections, we would expect a change in the size of these cells in that interval which must occur between the degeneration of this nerve ending and the reestablishment of a new nervous connection, a change which apparently does not occur. Because of the known potency of minute amounts of endocrine secretions, the most natural assumption is that this alteration has been effected through some 'hormonal' component of the plasma or lymph.

Rather interesting results have been secured by submitting these operated animals to adrenalin and to 'light and heat' stimuli. These experiments, although not as complete as could be wished, have been carried out on some half-dozen albinous and normal tadpoles into which grafts from the opposite type of animal had been made. The response or failure to respond in the case of host and transplant cells has been nearly identical. The tests were carried out from twelve hours to three days subsequent to the operation and supply additional evidence that the effective force causing a response of these cells to stimuli is the direct application of the internal secretory substance to the cell bodies and is not effected through the motor sympathetic endings, since it is probable that any nerve ending severed as it is from the nerve fiber would have degenerated in this interval, and even after this lapse of time new connections could hardly have been established.

Some rather significant evidence has been secured on the formation and migration of the epidermal melanophores in the transplants. There invariably occurred an early migration of the epidermal melanophores from the normal host into or over the albinous transplant. In a period of a few hours the sulcus intervening between the two types of skin would become filled with a dense mass of melanin-rich cells in which the pigmented epidermal cells could not readily be distinguished from the true melanophores. In twelve hours, or even less, growing points would appear from this confused scar tissue which invaded or

became incorporated gradually into the albinous graft. In this invading tissue, and indeed in those parts of the albinous graft which clearly had not suffered an invasion by the epidermis of the host, epidermal melanophores could readily be distinguished which curiously displayed, for a considerable interval, a state of great contraction. In the end the albinous transplant was as abundantly supplied with melanophores as was the normal epidermis. Although the free pigment of the albinous graft increased more slowly than did the epidermal melanophores, yet this too ultimately exhibited a normal condition. Thus, after an interval not exceeding two months, both the free pigment and the epidermal melanophores of the albinous transplant had assumed the characteristics typical of the epithelium of the normal host.

The sequence of changes in a dark graft to an albinous host is of a quite different nature from the alternate just described. Although there appeared to be a slight reduction in both the amount of the free pigment and the number of the melanophores of the epidermis, yet these changes were of a minor nature. No extensive migration of the melanophores from the normal transplant where they were thickly congregated into the sparsely supplied surrounding albinous epithelium occurred, nor by any other process did any great reduction in the number or pigment content of the transplanted melanophores take place during the interval that the animals were under observation (two months). These cells, once formed, appear to retain their integrity. This would appear to be the correct interpretation of the non-effect upon the pigment cells of hypophysectomy in the midlarval stages (Adler).

The effect of various diets upon the pigment cells

We have just shown by the skin exchanges that the atypical physiological state of the xantholeucophores and epidermal melanophores of the albino is directly referable to an alteration in the tissue juices which in turn is probably of an 'hormonal' nature. If, then, this endocrine disturbance which is inaugu-

rated by an hypophysial deficiency leads to an upset in the pigmentary system, will it not be possible with the proper endocrine diets to abort or ameliorate this derangement? The possibility appears the more probable since the general growth effects inherent in the loss of the buccal hypophysis can be prevented by the administration of the beef glandular lobe, as will be shown later.

Of the endocrine substances which have been fed, glandular lobe and neural lobe (with the pars intermedia) of the hypophysis, adrenal cortex, adrenal medulla, and liver, one only—the neural, or posterior lobe—has caused a formation of epidermal melanin approaching the normal, while none have reduced the xantholeucophores to a normal state of contraction.

It was early seen that those specimens receiving the posterior lobe of the pituitary exhibited a darker appearance than the albinous larvae fed upon the other fresh glands (figs. 46 to 51). Although expressed feebly at first, this depth of color became more pronounced as the feeding and growth progressed. So pronounced did it become after five months of this diet that there would have been some uncertainty in identifying these as hypophysectomized specimens save for the fact that legs did not develop. An examination of the living animal with the binocular reveals that although the xantholeucophores are typical of albinos, i.e., are fully expanded, there has been a great replacement of the epidermal melanin. This replacement was readily confirmed by a study of whole mounts of the epidermis which reveal that both components of the epidermal melanin have been increased (compare figs. 21 to 23 with figs. 14, 20). The free pigment is not, as in the typical albino, distributed in diffuse aggregations, but is found in considerable amounts in all of the superficial epidermal cells. The epidermal melanophores also show a depth of color never attained in the typical albino, as is most clearly shown if the processes of the broadly expanded melanophores (a condition readily obtained by the death of the animal) of the posterior-lobe-fed albino be compared with those of the albinos on any other diet.

Not only are the melanophores darker in the albinos receiving posterior lobe, but the number of these cells is also increased (table 1). Instead of averaging but thirty-eight to the unit area, they average in excess of fifty to this unit area (0.36 of a sq.mm.). Thus both in number and pigment content the epidermal melanophores of these animals are intermediate between those of the normal specimens and the typical albinos.¹⁵

It is not on the structural side alone that we find changes with a posterior lobe diet. The epidermal melanophores of these posterior-lobe-fed specimens uniformly exhibit a great and persistent contraction; they are uniformly either rounded or, at most, slightly irregular in shape, appearing as intense dark dots in all areas of the dorsal epidermis (fig. 21). Moreover, this contraction is persistent. After days of the substitution of another diet, it still persists; indeed, under the most potent stimulus—light and heat—for causing an expansion of the epidermal melanophores of the albino, a considerable interval must intervene in which neural lobe is not fed in order to obtain a decisive expansion of these cells.

It is curious that no increment in the epidermal melanin was produced in the *unoperated* tadpoles with a diet of fresh posterior lobe. Not only did no pigmentary increment result, but the contraction of the epidermal melanophores so typical of albinos on this diet did not regularly occur, or at most was but transitory.

Of the other substances which have been fed, none have appreciably affected the epidermal melanophores. One, namely, liver, has produced unmistakable differences, however, in the appearance of the xantholeucophores as contrasted to the albinos upon other diets. This effect is not expressed in any alteration in the usual extreme expansion of these cells, but rather in their color. These specimens do not show the golden sheen of the anterior or posterior lobe or adrenal cortex fed animals, but exhibit a chalky whiteness especially noticeable in the gill regions (figs. 46, 48), an appearance probably due to a reduction in the lipochrome content of the cells.

¹⁵ The author has not supplied albinous larvae with posterior-lobe extracts. It is apparent from Allen's work, however, that if they are potent in causing a melanin increase, they must be fed for a considerable interval of time.

The responses of the chromatophores to various physiological and pharmacological agents

It has been shown that the pigment cells of both the normal and the albinous tadpole are influenced by the internal secretory system. Since the endocrine system is abnormal in the albino, it is logical to inquire whether the atypical pigmentary system of these specimens will react to various stimuli as does this system in the normal animal.

Although we can largely confirm Laurens' observations (made on *Amblystoma*) upon the irregular reactions of the epidermal melanophores, it will be recalled that in the unoperated tadpole the usual state of these cells under indifferent environmental conditions—diffuse light and a gray background—is one of two-thirds to full expansion. The xantholeucophores, on the contrary, are punctate or slightly expanded, while the deep melanophores are fully expanded.

The usual condition of the epidermal melanophores of an albino in our so-called 'indifferent' environment was one of complete, or nearly complete, contraction. The xantholeucophores, on the contrary, are broadly expanded.

If we now place a large albinous and a large normal¹⁶ specimen in the dark for a period of one to two hours at room temperature, we find certain well-marked changes take place. The epidermal melanophores of the normal, if not completely expanded, become so, but those of the albino being previously greatly contracted are unaffected. The xantholeucophores of the normal become punctate; these cells in the albino remain fully expanded. The deep melanophores of both animals become greatly contracted, the animals then displaying a 'transparency' typical of the contracted condition of these cells. A low temperature (0° to 15°) apparently does not increase the reaction to any extent, the epidermal melanophores of the albino surely not being affected.

¹⁶ As complete an analysis of the reactions of the epidermal melanophores in the young normal tadpole as could be desired has not been made. The reactions here described unless otherwise stated apply only to large larvae at a time shortly preceding metamorphosis (in the normal).

If we now subject these large animals to the opposite environmental conditions (direct sunlight, a white background, and a high temperature—33° to 35°), we see a striking change take place. The epidermal melanophores of the normal frequently gradually contract and may ultimately after an hour or even less at times assume only a slightly expanded condition (fig. 19). These cells in the albino, on the contrary, invariably expand, ultimately exhibiting a fully expanded condition (fig. 20). There is thus frequently exhibited a reversal in the reaction of these cells in the large specimens of these two types of animals,¹⁷ a reversal not evinced to its fullest extent with any stimulus tried by the author save that of heat combined with direct sunlight. Either extreme heat or light evokes this reaction to some extent, the relative importance of each factor, however, being difficult to determine. A normal specimen, if placed in the dark at 30° to 35°, may exhibit after two to three hours partly contracted superficial melanophores. If the temperature becomes normal, these cells expand; if the temperature is maintained and sunlight used, further contraction not infrequently takes place. An albino, if placed in the dark at 30° to 35°, exhibits in two

¹⁷ One of the most striking features of the experimental work upon the epidermal pigment cells of the albinous and normal larvae has been the invariable response of these cells in the former as contrasted to their uncertain response in the latter. Of the very considerable number of albinous larvae which have been subjected to the stimulus of 'light and heat' a response, one of expansion, has invariably been exhibited by the epidermal melanophores. In contrast to this stands the variable response of these cells in the normal larvae. Because of the influence which the internal secretory glands have upon these pigment cells, this invariable response in the albino would appear to be of considerable significance, since certain of the endocrine glands in this type are structurally and also presumably functionally deficient. Consequently, we could expect that the response of these cells in this form would be more certain than in the normal where these cells are under the variable influence of the internal secretory products.

In 1919 (Proc. Soc. Exp. Biol. and Med., 44-1419) I called attention to the opposed reaction of the epidermal melanophores in the albinous and normal larvae. The experimental work upon which this description was based was carried out upon the older albinous and normal larvae and should have been so stated. These normal larvae very uniformly exhibited a contraction of their epidermal melanophores when subjected to 'heat and light' as contrasted to the expansion of these cells in the albino under identical conditions. Subsequent work has revealed a more variable response of these cells in the normal, but not in the albino.

hours partly expanded epidermal melanophores; if the temperature becomes normal, they contract to the usual condition; if the temperature is maintained and sunlight used in addition, they expand to the maximum. Thus we see that, although high temperature alone evokes the reaction, it is incomplete and slowly expressed, double the time being necessary. The same condition prevails if light alone is used. The reactions are evoked slowly and incompletely. Thus a combination of both light and heat is necessary to evoke the maximal response in the shortest time.

It has been mentioned that the xantholeucophores of the normal are uniformly nearly punctate in a dark-adapted animal. If the large normal animal be subjected to high temperature and direct sunlight, these cells become broadly expanded (fig. 19), never exhibiting, however, as great an expansion as invariably is presented by these cells in the albino. Thus we see that in the large normal animal there is frequently a reversal in the reaction between the 'interference' cells and the epidermal melanophores—one expands and the other contracts. Such a correlated opposite reaction of the pigment-cell groups no longer holds in the case of the albino, inasmuch as the xantholeucophores are always maximally expanded there. However, since under the usual environment the epidermal melanophores of the albino are almost maximally contracted, these two groups of cells are consequently usually in an opposite physiological condition, as is the case in the large normal larvae. We have already given evidence for our belief that the failure of albino xantholeucophores to react to stimuli is due to a tonic hormonal disturbance present in the tissue juices.

The deep melanophores with a white background and light expand; in darkness they contract; thus, in both types of animals their reaction is identical under all the environmental conditions employed by the author.

Specimens that have been thyroidectomized at an early stage, as is well known, exhibit no pigment deficiencies. Under the conditions of light and heat or of darkness, they react as do their unoperated brothers, with this difference: the reaction,

although of an identical nature, is more slowly evoked. In determining the possible cause for this tardy response, the work of Levy is particularly suggestive. This investigator has shown that the responses to adrenalin are more rapid if the thyroid has been stimulated or its extract injected. Although it has not been shown to what extent these responses of the pigment cells are referable either to the adrenal or directly to the sympathetic nervous system, still the slower responses of thyroidless larvae may well be of considerable significance in view of Levy's work. Furthermore, since pigment cells are formed and react in the thyroidless specimens in an identical manner with those of the normal, it is apparent that neither their atypical formation nor reaction in the albino can be referred to the atrophy of the thyroid effected by the hypophysectomy.

It has been shown that the epidermal melanophores of the posterior-lobe-fed hypophysectomized specimens exhibit a uniform state of great contraction, a contraction that persists for an extended period after the substitution of some other diet. Will these cells then respond to light and heat as do those of the typical albino?

It will be recalled that in the typical albino these cells, unlike the xantholeucophores, are sensitive to brilliant illumination coincident with increased temperature, undergoing an expansion. But the epidermal melanophores of the posterior-lobe-fed albinos stubbornly maintain their contracted condition with this stimulus and appear incapable of responding. Lest it be supposed that the reaction of these cells has approached that frequently exhibited by normal larvae, one need only to try their response to complete darkness where normal behavior would give us full expansion. Under all circumstances, their contracted condition is maintained just as is the corresponding persistent expansion of the xantholeucophores in these hypophysectomized larvae.

It was highly interesting to note that an abrupt change in the diet of the posterior-lobe-fed albinos, a substitution of a liver diet, led gradually to a resumption of the response characteristic of the typical albino. After an interval of one week in which they have received no nervous hypophysis they become

definitely expanded (fig. 22) when subjected to heat and direct sunlight. This response becomes more and more marked, until after five weeks we have an almost typical response (fig. 23). The xantholeucophores in these animals as in the typical albino remain broadly expanded under all environmental conditions. Posterior lobe feeding, then, which gives us an epidermal melanophore system which approaches the normal in melanin content, does not permit these cells to exhibit normal responses to light and heat. It merely maintains them in a refractory and contracted state, and when the feeding is stopped they exhibit the typical physiology of the albino.

The stimuli to which the two types of larvae have thus far been subjected are such as they might meet in the extremes of their normal environment. There is yet another class of stimuli which may be employed, namely, pharmacological agents, in which the larvae may be directly immersed. By this means we may be able to secure further evidence in regard to the endocrine locus responsible for the peculiar functional state of the chromatophore groups in the albino. Of the substances employed (pars intermedia emulsion, pituitrin, adrenalin, pineal gland emulsion), the first—pars intermedia emulsion¹⁸—from the profound pigmentary changes which it induces in the albino, especially merits attention. Atwell has called attention to the darkening of albinous larvae when placed in emulsions or extracts of this substance and has noted the response—one of expansion—of the deep melanophores to this treatment, a response which led him to refer the usual picture of albinism to a contraction of this melanophore group, and conversely the darkening of these treated albinous larvae to the expansion of these cells. We can fully confirm Atwell's observations as pertains both to the darkening of these larvae and the complete expansion of the corial melanophores when placed in the pars

¹⁸ The pars intermedia substance was secured by carefully clipping off pieces of this glandular substance from the subjacent neural lobe, as described by Herring ('14) and Atwell ('18). After drying on a warm plate the powdered substance was triturated with the desired amount of tap-water.

intermedia solution.¹⁹ Such a darkening clearly occurs, but an attendant color change was unnoted by Atwell. These larvae not only become darker, but they almost completely lose their metallic iridescent appearance so characteristic of the picture of albinism. As has been previously stated, this metallic effect is due to the broad expansion of the xantholeucophores, an effect which is displayed in its full beauty only because of the deficiency in the epidermal melanin which characterizes albinos. As might be surmised, then, these 'interference' cells have suffered a great contraction, causing an almost entire loss of the metallic-like albinous picture. Still another pigment change is wrought by the pars intermedia solution. The epidermal melanophores undergo expansion, ultimately assuming the expanded condition typical of the normal animal under 'indifferent' environmental conditions. It is thus seen that the pars intermedia emulsion can be described as bringing about a normal physiological state in this atypical pigment system of the albino and, as will be later pointed out, aids us in determining the endocrine locus responsible for this pigmentary disturbance.²⁰

When large albinous or normal specimens are placed in an adrenalin²¹ solution, serious respiratory and cardiovascular symptoms develop. The respiration becomes labored and slowed and the heart beat decreases in rate. Although more active for a few minutes, they soon become sluggish and rest in any position. Gradually the gills become flushed and it is noted that

¹⁹ Two strengths of pars intermedia solutions have been used. One made by triturating 10 mg. of the dried substance in 100 cc. of water, the other by triturating 10 mg. in 300 cc. of water. Both solutions produced identical effects, but at a different rate. The larvae would later succumb when left in the stronger solution for a period of one and a half hours or longer.

²⁰ The immersion of larvae in Pituitrin (Parke, Davis & Co.) has produced neither constant nor pronounced changes in the pigmentary system even when used in strengths sufficient to cause the serious distress or death of the animals. An emulsion of the posterior lobe (less the pars intermedia), even when used in strengths greatly in excess of that employed with the pars intermedia, appears not to evoke this reaction. Solutions of hypophysial colloid cause no pigmentary response.

²¹ Adrenalin (Parke, Davis & Co.) has been employed in strengths of 1 to 20,000 to 1 to 80,000. The stronger solutions (1 to 20,000 and 1 to 40,000 in amounts of 10 to 40 cc. per animal) have been of more value, and attention is called only to the responses evoked by these strengths.

the entire animal appears somewhat translucent. While evoked to an extent by the lower dilutions, these effects are much more clearly expressed with the stronger dosages. If the animals be examined with the binocular at five-minute intervals, the progressive alterations in the pigment cells can be followed. Especially uniform and particularly well pronounced is the contraction of the deep chromatophores. This contraction is the most important factor in the translucent appearance which follows the administration of adrenalin.

In adrenalin treatment the superficial melanophores are also affected, those of the albino more uniformly than those of the normal. In the albino these cells gradually expand and after an average period of forty to forty-five minutes a very changed state is evident. The picture presented is the reverse of that which obtains normally. Instead of these cells for the most part being in a contracted condition, they now show a state of expansion. Although only a part of this cell type is completely expanded, the percentage of completely expanded cells has definitely increased and all exhibit a more expanded state than before treatment. In the large normal animal the response of these cells when a considerable amount of fluid is used (40 cc. per specimen) is one of expansion. If only partly expanded, the processes soon present their maximum size; if already completely expanded, this condition persists. With a small amount of fluid (10 cc. per animal) these effects are not so pronounced and, curiously, are not infrequently reversed.

The third group of pigment cells, the xantholeucophores, also respond to adrenalin. In the albino the surface layer of these cells is the first to withdraw its processes, a response which may or may not extend to their more deeply placed brothers. Indeed, not all of these cells of the superficial stratum are affected, for here and there are interpolated areas of uncontracted cells, and a maximum contraction of all these cells has not been secured in the large albino, save with a lethal dose. In the normal, these cells react in a most varied manner, their reaction, as with the epidermal melanophores, apparently depending upon the amount of fluid used. With the larger amount there

is usually a contraction, although sometimes preceded by a preliminary expansion. With the smaller amounts these cells usually expand, there then being presented in the more favorable cases xantholeucophores of approximately the same size, both in the albino and in the normal.

The deep melanophores of both albinous and normal larvae contracted by a solution of pineal glands (two beef glands in 150 cc. water), the same transparency being exhibited as after adrenalin.²² This contraction of the deep melanophores, which, as pointed out, can be produced by darkness as well, somewhat intensifies the albinous appearance of true albinos, but its extreme effect in no case renders normal larvae albinous in appearance. These facts are of service in the evaluation of the relative significance of the various groups of pigment cells in producing the picture of albinism.

The anatomical and physiological findings which have been detailed may now be reexamined in order that we may be able to evaluate the relative importance which the various components of the pigmentary system have in creating the picture of albinism. Any explanation of structural or functional causes for the albinous appearance must keep clearly in mind the arrangement of these pigment cell types. It will be recalled that the deepest group of pigment cells—the corial melanophores—are overlaid by double strata (in most regions of the body) of xantholeucophores, which in turn lie beneath the epidermal pigment formed by the double component of free melanin and epidermal melanophores. With this arrangement in strata it is obvious, then, that a decrease in the color depth of an overlying stratum will exaggerate the effect of the subjacent one, or conversely an increase in the color depth of a stratum will proportionately decrease the color effect of those lying beneath. The decrease in pigment which obtains in the superficial pigment layer (epidermal) of the albino then exaggerates the chromatic effect of the abnormally expanded subjacent strata of the

²² With neither of these agents was the extreme transparency gained which McCord and Allen describe. Indeed, it is difficult to conceive how such an extreme effect could be produced with the very considerable amount of melanin which the epidermis of these specimens contains.

xantholeucophores, whose abnormal expansion in turn masks or minimizes the effect of the underlying layer, the deep melanophores. The mere arrangement which obtains, together with the physiological and structural modifications met in the albino, tends to exaggerate the effect of the 'interference' cell layers, and to minimize the rôle of the melanin-containing cells (the epidermal melanin being structurally deficient, the deep melanophores being masked by the overlying, broadly expanded layers of xantholeucophores), hence the color of the albino is characterized by its iridescent metallic nature. This effect is different from the transparency displayed by dark adapted normal larvae or those under pineal or adrenalin treatment, procedures which contract the deep melanophores and, since the xantholeucophores are unexpanded, enable this effect to be seen in a general lightening of tone (translucency) of the larvae.

It is possible to still further test the validity of this reasoning by examining the results of our experiments in which larvae were submitted to various stimuli (environmental and pharmacological) which brought about changes in the components of one or more of these pigment cell layers. A pronounced darkening of the albino was caused by immersion in *pars intermedia* solution. This change, however, does not permit us to analyze the rôle played by the various pigment strata, since all are affected (it will be recalled that the deep and superficial melanophores expand and the xantholeucophores contract, the epidermal free pigment of necessity not being changed). Yet by applying a test by which the pigment cells, save for the xantholeucophores, undergo the same change as with *pars intermedia* emulsion, we can determine whether the expansion of the deep and superficial melanophores has materially contributed to this darkening and loss of the metallic tone.²³ Such a test is afforded by placing albinous larvae in intense light when it is seen that they do not materially darken in color, although the deep melanophores may have been previously contracted. The translucency resulting from a maximal contraction of these cells has pre-

²³ The epidermal melanophores are so scanty in number and pigment content that their expansion *a priori* could have little effect.

viously been noted. It thus appears evident that the rôle of the deep melanophores in the formation of this picture of albinism is of no great importance. In fact, they are so completely masked in the body region in the albino by the overlying, broadly expanded xantholeucophores that from the anatomical arrangement alone it would be apparent that in *R. boylei* they could play no significant rôle in the formation of this picture, and most certainly not the primary rôle as maintained by Atwell in *R. sylvatica*.

It is also possible to test the relative significance of the diminished pigment content of the epidermis in the formation of this albinous picture. Attention has been called to the partial replacement of the pigment deficiency which takes place with a posterior lobe diet, and it was seen that these larvae became distinctly darker in color than the albinos not supplied with this gland. Or again, in the skin exchanges, the normal graft, whose xantholeucophores become broadly expanded, nevertheless is decidedly darker than the albinous host. This color depth can be referred in both cases in part to the color directly contributed by this epidermal melanin and in part to the masking of the underlying xantholeucophores by this pigment. It thus seems clear that the anatomical factors chiefly involved in the picture of albinism are, first, the broad expansion of the xantholeucophores; second, the diminution in the epidermal melanin which permits full display of the refractive xantholeucophores.

The greatest interest attaches to the determination of the endocrine locus responsible for the altered structural and physiological state of the pigment cells. Attention was called to the fact that the feeding of neither anterior lobe, adrenal cortex, adrenal medulla, nor liver deepened the color of the albino. On the other hand, a diet of posterior lobe (and associated pars intermedia) materially darkened the albino—an effect immediately referable to a partial replacement of the epidermal melanin. Also the immersion of the larvae in a solution of pars intermedia alone, of all the endocrine extracts employed, produced a normal physiological condition of the chromatophore system. It would

thus appear from these responses that the extracts or diets of the intermediate and posterior lobes of the pituitary were intimately concerned in this pigmentary fault. Corroborative evidence is supplied by a study of the structural malformations occurring in the endocrine glands of the albinous and the partially hypophysectomized larvae. For, as will be detailed in a subsequent section (4), the neural hypophysis, thyroid, epithelial bodies, adrenal cortex, and adrenal medulla suffer definite and often profound structural and size alterations in the albino. In the partially hypophysectomized tadpole, however, whose pigment system may suffer a profound disturbance (indeed, the picture of albinism approaching that of the true albino), none of these internal secretory organs save the posterior lobe and the associated pars intermedia exhibit significant structural defects.²⁴ The evidence thus points to a deficiency in the secretion formed by either the posterior or intermediate lobes of the pituitary or by the 'interaction' of these two intimately associated components of the pituitary as being responsible for the pigmentary upset in the larvae.²⁵

²⁴ Further evidence freeing the thyroid from responsibility for this pigmentary disturbance comes from the thyroidectomized specimens whose pigmentary system is not at fault.

²⁵ Indeed, the pigmentary system in the frog, at least, appears to be very sensitive to hypophysial disturbance. This is shown in a striking way by one specimen of a considerable number (seventy) of tadpoles which suffered the extirpation of their pineal glands in an early larval stage. In this operation the aqueduct of Sylvius of one specimen was inadvertently injured, an injury leading to its complete occlusion. This apparently induced an internal hydrocephalus of the III and lateral ventricles with the consequent inevitable pressure upon the pituitary, sections revealing a flattened gland slightly reduced in size. The specimen exhibited unmistakable albinism, a condition unquestionably referable to the compression of the pituitary gland, since it is certain that this pigmentary disturbance was not due to the loss of the pineal body, for, as has been shown by Laurens ('13), the loss of this gland produces no fault in the pigmentary system, ample confirmation of which has been made in this laboratory.

3. GROWTH DISTURBANCES

Little doubt can be entertained but that the pituitary gland plays a significant rôle in the regulation of growth. Observations on clinical material which have been elucidated and extended by experimental operative work, more particularly by that of Paulesco and Cushing, have shown rather clearly that a hypersecretion of this gland results in an overgrowth of the skeletal system (giantism and acromegaly), the opposite picture of a pituitary deficiency being seen in dystrophia adiposogenitalis.

Clear as such findings appear to be, it seems the more enigmatical that the administration of this gland has failed to elicit a definite growth accelerative response, indeed the response most uniformly evoked being one of retardation. The evidence so far presented is in many cases at least of such an equivocal and uncertain nature as to come well within the limits of experimental error and to even be subjected to an opposite interpretation. This can be said not only of the feeding experiments of different investigators, but of single investigators as well, and only to the latter do we need to address our attention for a moment. Aldrich ('12), from the administration of anterior lobe to pups, concluded that growth was not stimulated nor retarded, but later concluded from work on white rats that growth retardation occurred (Aldrich, '12). Robertson, in the most elaborate work which has yet appeared upon this subject and who has not only used large numbers of animals of the same species (white mice), but who has also applied the valuable adjuvant of refined statistical methods to his data, at first concluded (Robertson, '16), "The administration of 0.125 gms. per day per animal of fresh anterior lobe pituitary tissue to mice, beginning at 4 weeks after birth (conclusion of the second growth cycle), leads to retardation of growth during the earlier portion of the third growth cycle, between the 6th and 20th weeks. In the latter part of the third growth cycle, however, from the 20th to the 60th weeks after birth, the growth of the pituitary-fed animals is markedly accelerated, so that they

not only catch up to the normals, but actually, at about one year of age, come to surpass the normals in weight." Later he says (Robertson, '19): "The computation of the deviations in terms of probable error units shows that the effect of the dosage of pituitary tissue administered upon the growth of the male animals was of uncertain significance, since the observed deviations were only from one to two times the probable error. That the deviations from the normal nevertheless were real and due to the administration of the pituitary tissue is evidenced by the much greater effect of the same character upon the female, consisting of a retardation during the earlier stages of growth. In both males and females the deviations from the normal after the 30th week are of indeterminate significance; that is, the growth curves of the normal and of the pituitary-fed animals are, so far as our estimates reveal, identical after seven months of age. Hence the preliminary retardation of growth has clearly, prior to the 30th week, been succeeded by acceleration." That a transient growth retardation in the young animal results from pituitary administration seems evident not only from the work of Robertson ('16, '19), but from that of Cerletti ('07), Sandri ('09), Aldrich ('12), Wulzen ('14). But that any subsequent acceleration which may be exhibited is due to anterior lobe administration is not clear. Indeed, it seems not improbable that such an acceleration is compensatory, such as Osborne and Mendel have shown to occur with an adequate diet after an animal has been stunted by undernutrition. This seems the more probable in view of Robertson's feeding experiments with Tethelin in which he was forced to abandon the view which he had previously strongly urged—that his alcoholic anterior lobe extract (Tethelin) supplied a growth-accelerating principle. These animals, indeed, exhibited a more pronounced secondary acceleration when the Tethelin diet was withdrawn than when it was continued.

The principle has been formulated by Halsted that a 'physiological deficit' is essential for successful organ transplantation. From analogy it seems not improbable that if a physiological deficit could be created in experimental animals, they would

be able to 'utilize' the growth-maintaining ('accelerating') principle of the administered anterior-lobe substance as they could not do were this deficit not existent. If, then, the animals suffering from such a deficit exhibited a pronounced retardation in growth and if in other animals this retardation could be prevented by pituitary administration, we would obtain a combination of conditions giving a pronounced effect from such a dietary régime. Such indeed is the case in the hypophysectomized (albino) frog tadpole, as has previously been pointed out by the author (Smith, '18). The growth response to anterior lobe administration in such animals, as compared to the normal, is greatly magnified, since, 1) the hypophysectomized frog tadpole grows at a slower rate than the normal (Smith, Allen) and never attains, or attains only after an extended period, the size of the normal tadpole (Smith) and, 2) the operated tadpole supplied with bovine anterior lobe not only exhibits a normal growth rate, but, due to the persistence of the larval condition and the consequent extension of the larval growth span, ultimately attains a size notably in excess of the normal. By this sensitiveness of the albino frog tadpole a clear response to the growth-maintaining principle of the pituitary has been secured.²⁶

The ease of securing such operated material, its prolonged survival after buccal hypophysectomy, and the magnified response of the albino frog tadpole to anterior-lobe administrations all combine to make this material of singular value in testing various hypophysial extracts and residues for the presence or absence of the growth-maintaining ('accelerating') principle. Such material alone affords an ideal way to analyze physiologically the various hypophysial substances which have been administered in order to determine the rôle of this gland

²⁶ It will be quite evident why the term growth-'maintaining' substance is used in comparing the growth rate of the albino larvae with the growth rate of the normal. The growth rate of the albino is not accelerated by the administration of anterior lobe as compared to the normal; their growth rate is maintained. Such a diet, however, clearly accelerates the growth of the albino tadpole as compared to their albino fellows on liver diet.

in growth.²⁷ It is obvious that a determination of the presence or absence of a growth-'accelerating' principle in an extract or fraction of this gland can hardly be made with the normal animal when the administration of the fresh gland does not call forth such a response.

The growth rate of the albino; the effect of the administration of various hypophyseal substances upon this growth rate

In order to discuss the deviations from normality of the growth rate of the hypophysectomized tadpole and the effect of the administration of fresh bovine anterior lobe and its extractives and residues upon these deviations, it will first be necessary to examine the growth rate of the normal unoperated tadpole. The growth curve of a normal tadpole with a liver-lettuce diet consists of three phases, the first two indistinctly separable, the third more pronounced. An early transient and not clearly marked period of slow growth, which perhaps should receive even less emphasis than has been accorded it (Smith, '18), shades insensibly into a protracted second phase of rapid growth during which the tadpole attains nearly its maximum size, and which is in turn terminated rather abruptly by the advent of metamorphosis (fig. 1). The administration of bovine anterior lobe neither alters the nature of this curve nor produces quantitative results of unquestionable significance, although there appears to result in most cases from this diet a small increment in size and a somewhat earlier completion of metamorphosis.

The growth curve of albinous frog larvae with a liver-lettuce diet differs in character and in magnitude from that of the normals (fig. 1). I have described these changes previously (Smith, '18), and later work has amply confirmed them. A retardation in growth following the appearance of albinism is unquestioned and becomes progressively greater until approximately a 30- to

²⁷ Caselli ('00), whole-gland glycerin extracts; Cerletti ('01), a centrifugalized aqueous-glycerin emulsion; Aldrich ('12), fresh desiccated non-defatted anterior-lobe substance; Aldrich ('12), fresh defatted anterior-lobe substance; Robertson ('16), fresh anterior lobe and his alcoholic extract, Tethelin, of the desiccated anterior lobe.

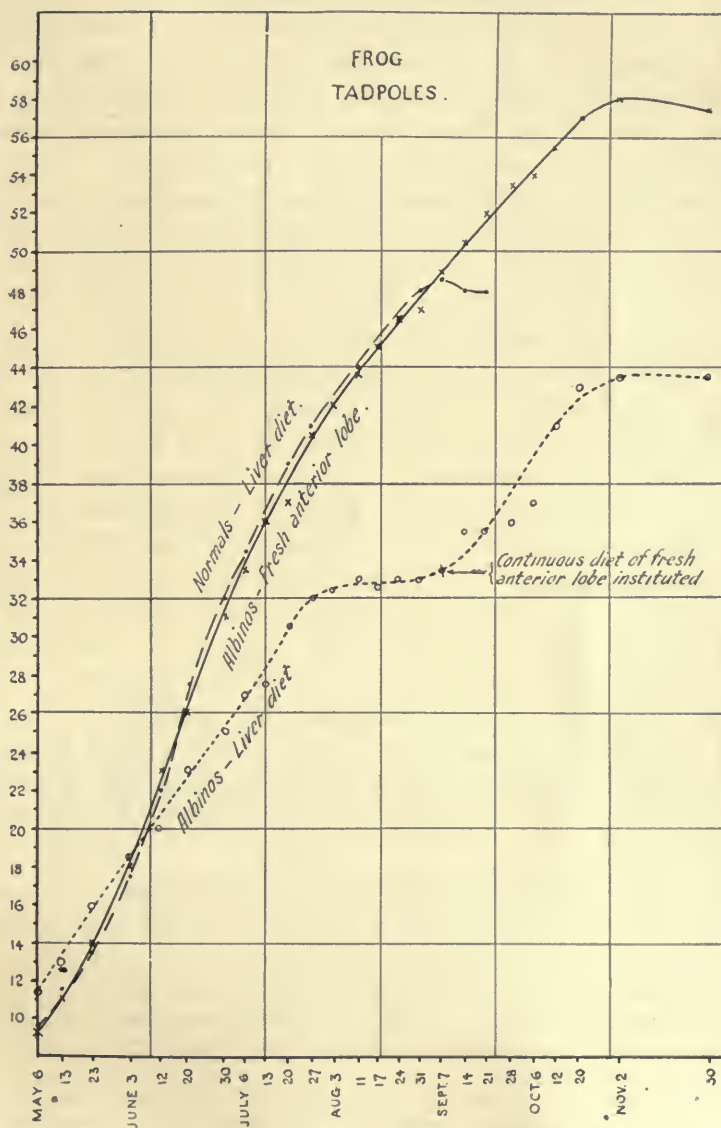


Fig. 1 Growth curves of normal frog larvae supplied with a liver diet, hypophysectomized frog larvae supplied with liver and with fresh anterior-lobe substance. The ordinates represent the total length of the larvae expressed in millimeters.

32-mm. stage (midlarval period) is reached. At this stage the retardation becomes extremely pronounced and an abrupt change in the direction of the growth curve ensues. So definite and so characteristic is this point that it appears to indicate a significant period in the development of these albinous larvae and hereafter will be designated as the 'critical point.' Both in time and stage of development this point has been coincident in the curves plotted from data secured in 1918 and 1919. From this 'critical point' the divergence between the curves of the normal and albinous larvae increases for a time, the curves later approaching each other again due to the onset of metamorphosis in the former and the continued growth of the latter.²⁸

It is now possible to inquire whether the administration of a 'substitution' or 'replacement' diet of fresh bovine anterior lobe will effect a normal rate of growth in these animals suffering from hypophysial deprivation. This can be answered for the frog tadpole in the affirmative. The albinous frog tadpoles supplied throughout their growing period with a continuous diet of fresh anterior-lobe substance exhibit no significant growth deviation from the liver-fed normals (fig. 1, table 2). Since metamorphosis does not supervene, however, their growth extends beyond the normal larval period, and they consequently attain a size notably in excess of their unoperated normal fellows.²⁹ The alimentary régime of fresh bovine anterior lobe has then clearly supplied the morphogenic principle lost by buccal hypophysectomy.

It is conceivable that pituitary substance greatly in excess of the amount actually requisite to replace the growth principle

²⁸ It will be noted from the tables that after September 7th a continuous diet of fresh bovine anterior lobe was supplied the albinos whose curve of growth is here plotted. This apparently caused an abrupt rise in their growth curve. The growth curves of a group of albinos supplied throughout their life-span with a liver diet are shown on page 18 (Smith, '18).

²⁹ The final and rather abrupt termination in growth exhibited by the albinous specimens might conceivably be due to one of at least two factors: 1) a self-limiting growth factor inherent in every organism; or, 2) a seasonal growth rhythm. That this is not attributable to the latter is shown by a group of albinous frog larvae which survived for seventeen months. No resumption of growth occurred in the second season; rather, they became progressively more lethargic, the anorexia increased, and death finally supervened.

TABLE 2
Table showing the maximal, minimal, and average (most commonly occurring dimension) lengths of the frog larvae on which the accompanying growth-curves are based

BUCCAL COMPONENT OF HYPOPHYSIS ABLATED												THYROID ABLATED	NORMAL
DIET OF GLANDULAR LOBE OF HYPOPHYSIS													
Date	Liver diet	Fresh glandular lobe	Fresh glandular lobe May 6 to July 18	Fresh glandular lobe once a week	Alch. extract	Alch. residue	Aqueous extract	Aqueous residue	Colloid	Liver diet	Fresh glandular lobe	Liver diet	Liver diet
May 6 ¹	11-12 11.5	9	10-12 11	10-12 11	9	9	9	9	9	9-10 9.5	10-12 11	9-10 9.5	9-10 9.5
13.....	12-14 13	10.5-11.5 11	12-14 13	11-14 12.5	11-12 11.5	11-12 11.5	11-12 11.5	11-12 11.5	11-12 11.5	11-12 11.5	12-14 13	11-12 11.5	11-12 11.5
23.....	14-18 16	13-15.5 14	14-18 16	14-17 15.5	13-15 14	13-15 14	13-15 14	13-15 14	13-15 14	12-14 13	12-16 14	12-16 13.5	12-16 13.5
June 3.....	15-20.5 18.5	16.5-20 18	17-22.5 19	16-20.5 18.5	17-20 18	17-19 18	16-18 17	17-20 18.5	17-20 18	15-19 16.5	15-21 18	15-20 17.5	15-20 17.5
12.....	18-24 20	17-25 23	20-29 23.5	17-24 22	19-24 20.5	19-25 22	17-21 20	21-26 23	19-24 20.5	18-26 22	20-27 22	18-25 22	18-25 22
20.....	19-27 23	19-30 26	20-32 27	18-28 25	20-24 21	22-28 26	17-22 20.5	27-30 28	20-24 21	20-32 27	21-31 26	21-32 27.5	21-32 27.5
30.....	24-29 25	25-35 31	24-40 30.5	22-30 26	21-25 23	23-32 29	21-25 23	27-34 30	21-25 23	22-37 30.5	24-38 32	23-38 32	23-38 32

¹ First date of special feeding.

TABLE 2—Continued

BUCCAL COMPONENT OF HYPOPHYSIS ABLATED												THYROID ABLATED	NORMAL
DIET OF GLANDULAR LOBE OF HYPOPHYSIS													
Date	Liver diet	Fresh glandular lobe	Fresh glandular lobe May 6 to July 18	Fresh glandular lobe once a week	Alch. extract	Alch. residue	Aqueous extract	Aqueous residue	Colloid	Liver diet	Fresh glandular lobe	Liver diet	Liver diet
July 6.....	23-33 27	25.5-36 33.5	25-41 31.5	23-32 28	21-26 24.5	24-34 30.5	21-27 25	27-35 31.5	21-26 24.5	24-42 31.5	25-39 33	24-39 34.5	
13.....	25-35 27.5	28-40 36	28-44.5 35	26-33.5 30	22-28 25.5	29-40 32	23-29 25.5	28-31 34	22-28 25.5	26-46 37	26-40 35.5	26-45 36	
20.....	25-36 30.5	31-44 37	29-48 40	28-35 31.5	24-28 28	30-43 34	24-31 27.5	29-38 34.5	24-28 28	27-49 40	27-43 39	29-49 39	
27.....	26-38 ² 32	31-46 40.5	30-49 41.5	29-37 32	24-29 28	30-46 36	26-32 28	29-40 36	24-29 28	27-50 42.5	28-47 41	31-52 41	
Aug. 3.....	28-39 32.5	32-48 42	33-49 41.5	28-36 32.5	24-30 28	33-47 38	26-31 28.5	32-41 39.5	24-30 28	32-51 43.5	31-47 41.5	32-55 42	
11.....	28-37 33	32-51 43.5	33-50 42.5	27-37 32.5	25-30 28.5	32-47 40.5	28-33 29.5	30-42 40	25-30 28.5	32-52 45	31-51 41.5	32-56 44	
17.....	28-38 ² 32.5	32-54 45	34-52 42.5	28-40 33	26-31 28	34-47 41.5	27-31 29.5	31-45 42.5	26-31 28	35-54 46	31-49 43.5	34-57 45	
24.....	29-36 33	32-56 46.5	35-53 43.5	30-41 34.5	27-30 28.5	35-52 42	27-32 30	33-48 45	27-30 28.5	35-57 46.5	32-55 46	35-55 46.5	

31.....	28-36 33	32-58 47	35-53 45	32-42 36	27-31 28.5	36-54 45.5	27-35 30.5	32-50 46.5	27-31 28.5	38-58 48	33-56 48	39-52 48
Sept. 7.....	29-40 ³ 33.5	32-60 49	35-53 45	32-43 38	27-31 29	36-56 48	28-35 31.5	35-52 47	27-31 29	40-60 49	39-62 49	40-55 48.5
14.....	31-39 35.5	39-60 50.5	35-54 46.5	33-44 39	26-31 29	37-56 49.5	29-35 31.5	36-52 49	26-31 29	40-59 52	40-59 52	42-53 48
21.....	28-41 35.5	39-61 52	35-56 47.5	34-46 40.5		37-58 50	31-36 33	35-56 51.5		40-60 52.5	40-62 55.5	45-52 48
28.....	33-43 36	39-65 53.5	35-57 48	35-50 42		37-61 51.5	34-37 34.5	35-56 54		42-61 54	40-68 56	46-51 48
Oct. 6.....	31-43 37	39-64 54	35-57 48	36-52 44		37-62 52.5	34-37 35	36-57 55		42-64 55	40-69 57	
12.....	32-44 41	39-68 55.5	35-56 47	36-52 44.5		38-62 55		38-59 57		43-64 55	40-66 ⁴ 56	
20.....	34-48 43	40-69 57	35-57 47	36-52 45		44-62 57		54-61 58.5		43-65 56.5	40-66 56	
Nov. 2.....	34-52 43.5	44-69 58	38-56 47	36-57 47		44-62 57		56-59 58		43-67 59	40-67 58	
30.....	37-50 43	44-67 57.5		41-58 49						43-67 58	42-67 60	

² Subsequent development showed that the large measurement was from a partial albino.

³ Diet changed to fresh glandular lobe.

⁴ Several large specimens removed for special feeding.

lost by hypophysectomy has been supplied these albinous larvae exhibiting a normal growth rate, since it will be noted that this anterior-lobe substance was fed daily and formed their entire food supply aside from a small component of lettuce. This seems the more probable if the conception usually held in regard to 'hormonal' substances be correct, namely, that minute amounts are effective. But that this substance must be supplied continuously is clearly shown by three groups of albinos: 1) a group receiving anterior-lobe substance once each week, liver being fed on the other days; 2) a group receiving anterior lobe until an average length of 36 mm. (on July 18th) had been attained, a liver diet then being instituted; 3) a group (the converse of group 2) reared on a liver diet until they had attained an average length of 33 mm. (on September 7th), the liver diet then being replaced by anterior lobe.

The growth curve of the first group, those receiving anterior lobe once a week, although considerably modified, resembles that of the liver- rather than that of the hypophysis-fed group (fig. 2). Though the incidence of the early retardation is slightly deferred, nevertheless the 'critical point' appears in the usual position, though in a less pronounced manner. After a brief period of depression, the curve rises in a regular manner until the level of the normal is attained. Their curve thus resembles that of the liver-fed albinos, and the ultimate size attained by these specimens is intermediate between that of the anterior-lobe- and that of liver-fed specimens.

The second group, those receiving anterior lobe until July 18th, grew at a normal rate up to and for approximately two and one-half weeks past the time when the liver régime was instituted (fig. 2). A definite growth retardation then appeared, the specimens eventually attaining the size of their unoperated companions, but failing by some 10 mm. to reach the size of their operated mates supplied throughout their growing period with glandular hypophysis.

The specimens of the third group, those supplied with liver until they had attained a size of 33 mm., had suffered the usual early growth retardation and the pronounced midlarval slowing

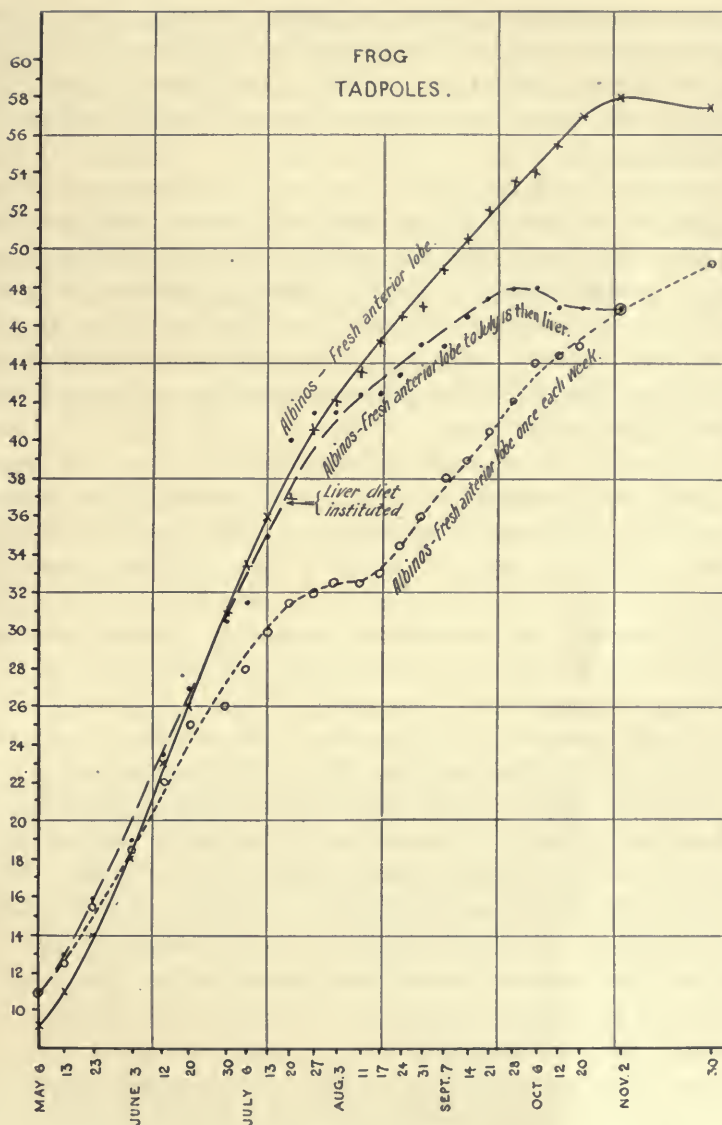


Fig. 2 Growth curves of hypophysectomized frog larvae supplied with a continuous diet of fresh anterior-lobe substance, anterior lobe once a week and anterior lobe from May 6th to July 18th, then liver. The ordinates represent the total length of the larvae expressed in millimeters.

invariably exhibited by albinos supplied with this diet. Following the substitution of a hypophysial for a liver diet, a normal growth rate is exhibited for a time, the albinos, however, not attaining the large size induced by continuous anterior-lobe feeding (fig. 1).

It seems clear from these three groups that neither an interrupted diet of anterior-lobe substance nor a full diet of this substance during merely the early or the late portions of the growing period will bring about a normal growth of the albinous larvae. It is indeed remarkable that albinous larvae, growing at a normal rate in response to anterior-lobe administration, will exhibit a growth retardation so quickly on the withdrawal of this 'substitution' diet.

It may then be regarded as demonstrated that the retardation in growth, resulting from the early removal of the epithelial hypophysis, can be ameliorated by a continuous diet of bovine anterior lobe, the growth curves of the albinous larvae thus treated showing no deviation from normality save by its continuance beyond the normal growing period. It will likewise be granted by the reader that such material is of peculiar value for determining the presence of the growth-maintaining principle in any hypophysial extractive or its residue. The experimental utilization of this material should show us whether the extraction of the fresh anterior lobe with boiling alcohol or boiling water removes or destroys this growth-maintaining principle—a test which can be provided with a double check, since both the extract and its residue can be fed to similar groups of albinos. To the group of four substances (aqueous extract and its residue, alcoholic extract and its residue) which were submitted to this test was added the hypophysial colloid occurring in the intraglandular (vestigial cleft) of this gland.³⁰

³⁰ In the preparation of the aqueous extract and its residue, the ground fresh anterior-lobe substance was extracted in a modified Bailey-Walker apparatus (Robertson) over boiling aq. dest. for forty-eight hours, five bovine anterior lobes being placed in each alundum thimble. After this prolonged extraction the substance was dried, powdered, and placed in vials tightly corked. Each group of specimens (20 to 24) received the dried residue from one and a half glands at a feeding. The clear, yellowish extract on evaporation yielded a dark, readily soluble amorphous

Obviously, since the aqueous and alcoholic extracts and the colloid were of slight or no nutritive value, additional food substance had to be furnished, but such food substances were only supplied after obtaining a maximum intake of the special glandular substance. After turning off the flowing water, the special substance was fed, one half-hour later the nutritional substances, liver and lettuce, being supplied. Twenty-four hours later, after removing all food particles, the process was repeated.

In the accompanying table (table 3) is shown the number of frog larvae at the commencement of the special feeding and at three subsequent periods. Although the number is relatively small, yet they were average-sized, carefully selected, healthy tadpoles free from injury or malformation. Work with a larger number was precluded, due to the difficulty of securing a sufficient amount of the special substances, 90 to 100 bovine hypophyses being used daily with even this number of specimens.

substance giving a lipid-like odor on burning. The extract from five glands was supplied each group of tadpoles (20 to 24) at a feeding. The feeding was done with sufficient rapidity to insure against an extensive solution of the solid substance, and from the rapidly initiated movements of the tadpoles it was certain that they were feeding directly upon this substance.

The alcoholic extract (Tethelin) was supplied by H. K. Mulford Company. In the feeding of this substance even greater care was exercised than with the aqueous extract, to guard against solution. After a period of a few seconds subsequent to the breaking of the vacuum-sealed tube, the tadpoles would be feeding upon the substance. Fifty milligrams (the extract from five glands) was given each group at feeding.

In the preparation of the alcoholic residue the method elaborated by Robertson for the extraction of Tethelin was slightly modified. Dehydration was carried out with 95 per cent alcohol instead of anhydrous sodium and calcium sulphates, since the addition of the salts would render the residue useless. Following dehydration, the tissue was thoroughly dried over an electric stove, then extracted in a Bailey-Walker apparatus with boiling absolute alcohol for forty-eight hours. The residue, freed from alcohol, and preserved in tightly corked vials, was supplied in amounts equal to one and a half of the fresh glands at a feeding.

The colloid was secured from the vestigial cleft, between the glandular and intermediate lobes. The amount obtained from the forty-five glands and administered to the twelve to fifteen tadpoles at each feeding varied considerably, but averaged 0.5 gram. Occasional very large pieces (0.675 gram) were found, many other glands revealing none. Usually it occurred as a firm, amorphous mass, molded by the shape of the cleft, now and then, however, gels of differing viscosity or even an aqueous-like substance was obtained. Before feeding, the amorphous masses were crushed and mixed with the more fluid portions.

Further, it was believed that if this number of animals should show an unequivocal response to hypophysial administration, it would furnish convincing evidence of either the presence or the absence of the growth-maintaining principle in these special substances—a belief which we feel has been amply sustained.

The growth curves of the albinous frog tadpoles supplied with either alcoholic or aqueous residues show the same maintenance of growth as is exhibited by their fellows fed with the fresh anterior lobe (figs. 3, 4). No 'critical point' appears; the three curves are coincident.

This is not the case with frog albinos receiving aqueous extract, Tethelin, or colloid (figs. 3, 4, 5). Their curves are quite similar to that of the liver-fed albinous larvae. The same early retardation is noted, the 'critical point' is well marked, but appears at a somewhat earlier period (twelve days) and at a somewhat smaller average stage (6 mm.) than their liver-fed companions. Following the critical point, some divergence appears in the three curves. The specimens supplied with aqueous extract exhibit a slight secondary acceleration in growth, but they do not attain quite the average length of their liver-fed mates. The 'Tethelin' group does not exhibit any secondary acceleration subsequent to the 'critical point,' while the colloid group exhibits an even greater retardation than its fellows supplied with Tethelin.

In order to further test the growth effects of hypophysectomy and its response to an anterior-lobe diet, a series of experiments similar in every way to those just reported upon were carried out with toad larvae. These larvae respond in general in the same way as the similarly treated frog tadpoles. The early retardation, the usual midlarval slowing ('critical point'), followed by slow growth, appears as in the frog tadpole (fig. 6). In this, my observations are somewhat at variance with those of Allen, who reports no retardation in the velocity of growth in hypophysectomized toads. Fuller reports may explain this essential difference in our results. I wish to emphasize, however, that the early retardation, the 'critical point,' and the later period of slow growth are not so distinctly shown in the

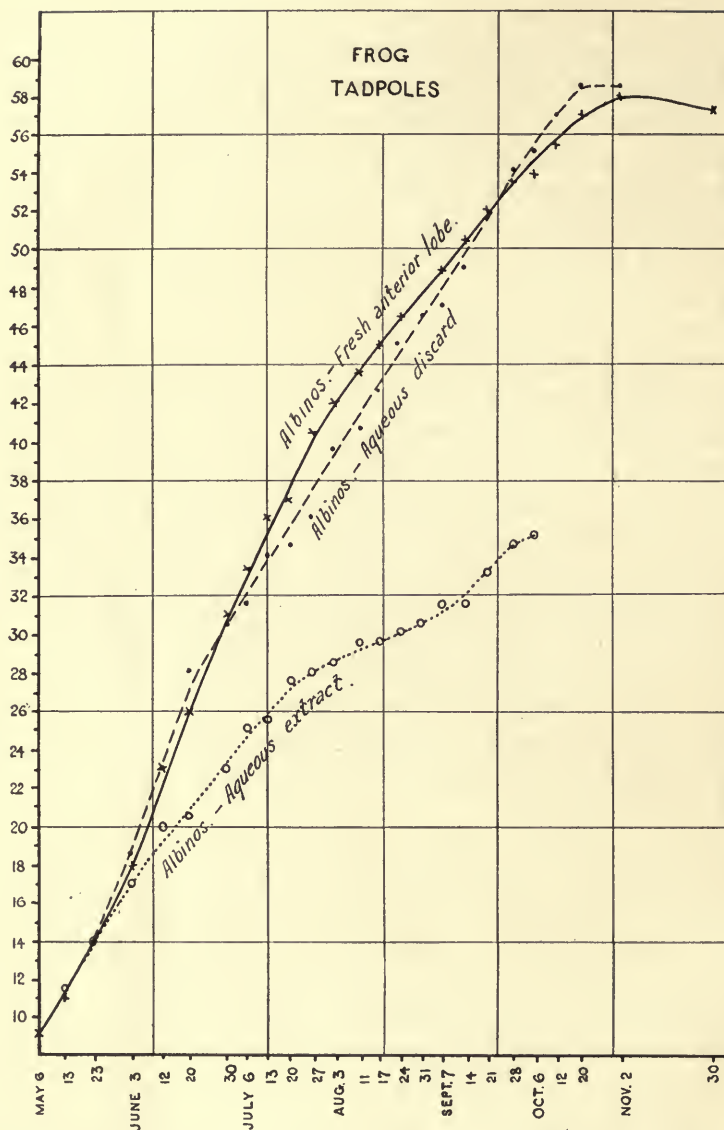


Fig. 3 Growth curves of hypophysectomized frog larvae supplied with fresh anterior-lobe substance, with aqueous extract, and with aqueous residue of the anterior lobe. The ordinates represent the total length of the larvae expressed in millimeters.

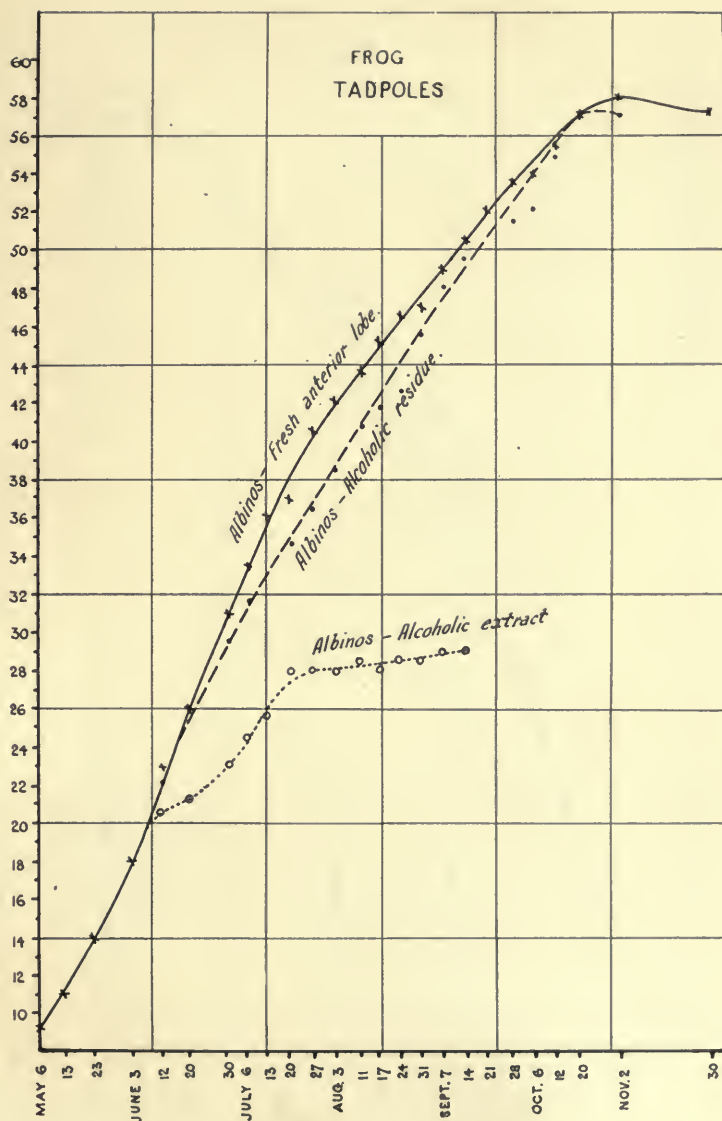


Fig. 4 Growth curves of hypophysectomized frog larvae supplied with fresh anterior-lobe substance, with alcoholic extract and with alcoholic residue of the anterior lobe. The ordinates represent the total length of the larvae expressed in millimeters.

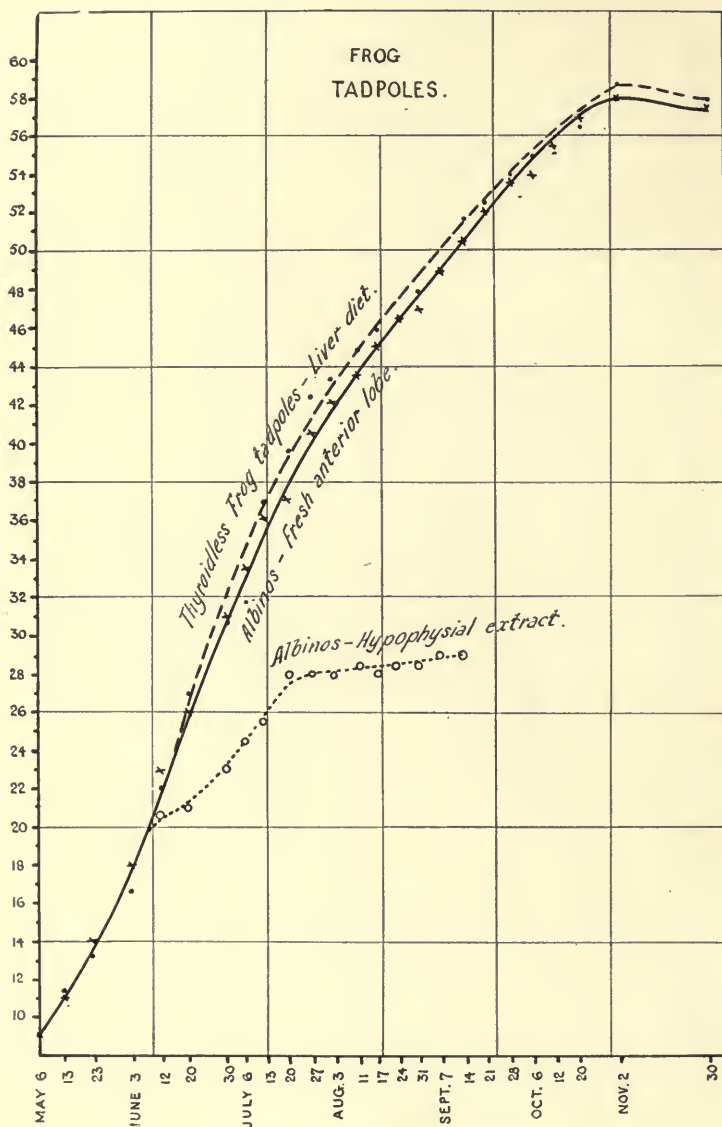


Fig. 5 Growth curves of thyroidectomized frog larvae supplied with liver diet, hypophysectomized frog larvae supplied with fresh anterior-lobe substance, and with hypophysial colloid. The ordinates represent the total length of the larvae expressed in millimeters.

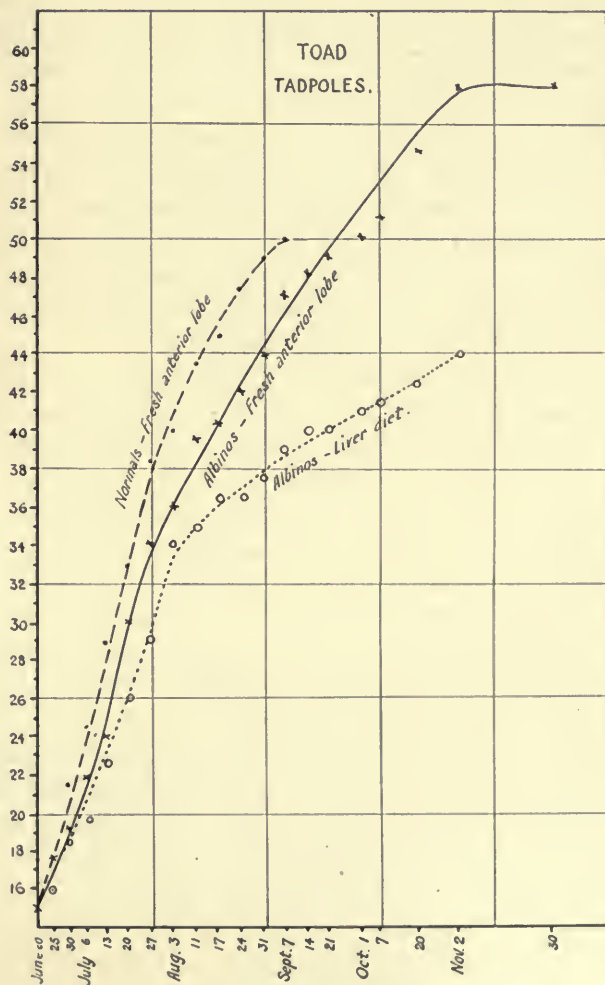


Fig. 6 Growth curves of normal toad larvae supplied with fresh anterior-lobe substance, hypophysectomized toad larvae supplied with fresh anterior-lobe substance and with liver. The ordinates represent the total length of the larvae expressed in millimeters.

toad as in the albinous frog larvae. This is also true of their response to a 'substitution' diet of anterior-lobe substance. The growth rate of those toad albinos supplied with an hypophysial diet does not quite equal that of their normal brothers.³¹ Neither has the administration of hypophysial extracts, residues, and colloid given as clear results as in the frog tadpole (figs. 7, 8, 9). However, an unmistakable growth retardation is shown by the albinous toad larvae supplied with the extracts and colloid, and the administration of the residues has caused a distinct acceleration in growth rate as compared to their liver-fed albinous mates. Table 4 gives the measurements upon which the growth curves of the toad tadpole are based, and table 5, the number of specimens used in the study.

From a survey of these groups receiving the various hypophysial substances and liver it is evident that they are separable, whether frog or toad larvae be used, into two distinct categories, both by the size attained and by the nature of their growth curves. On the one hand is the group formed by those larvae supplied with either the alcoholic or aqueous residues; their growth curves are identical with that of the fresh anterior-lobe-fed group; on the other hand is the group formed by the larvae receiving Tethelin, aqueous extract and colloid; these exhibit some variation in size, but their curves are similar in nature to that of the liver-fed albinos.

To what factor or factors is due, 1) the normal growth rate of the albinous larvae supplied with fresh anterior lobe and the hypophysial residues, and, 2) the retardation of specimens fed with liver, hypophysial extractives, and colloid? The normality of the growth rate exhibited by the specimens of the first division might conceivably be due to the superior nutritive value of the anterior lobe rather than to a morphogenic agent. If this was the responsible factor, however, we should expect as great a deviation in both the magnitude and the nature of the growth curves of normal tadpoles fed in this way. No such

³¹ It is to be noted, however, that the normal specimens received a diet of fresh anterior lobe, not of liver. A slight acceleration in the normal produced by this diet may account for the divergence in the two curves.

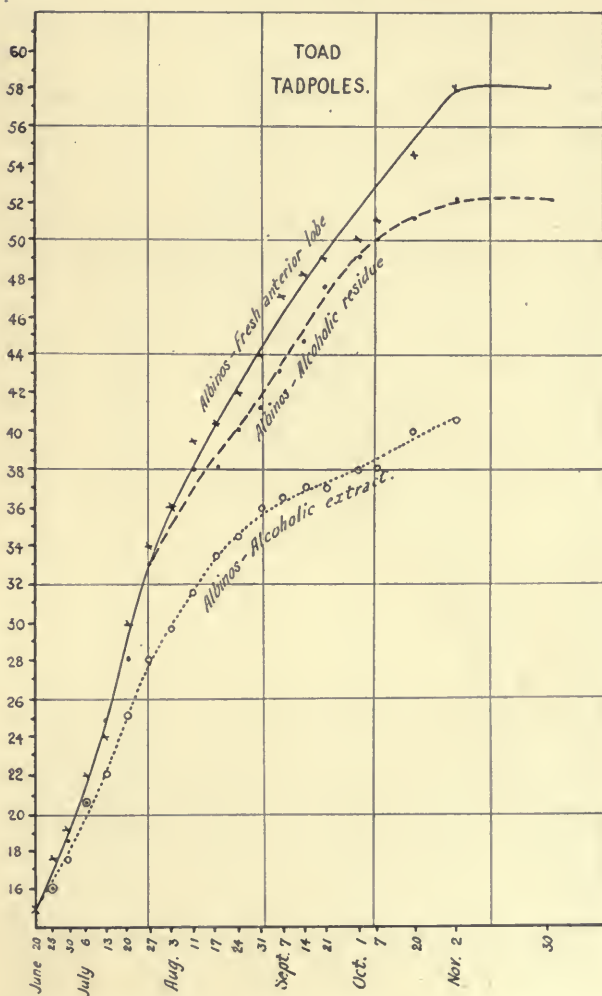


Fig. 7 Growth curves of hypophysectomized toad larvae supplied with fresh anterior-lobe substance, with alcoholic extract, and with alcoholic residue of the anterior-lobe. The ordinates represent the total length of the larvae expressed in millimeters.

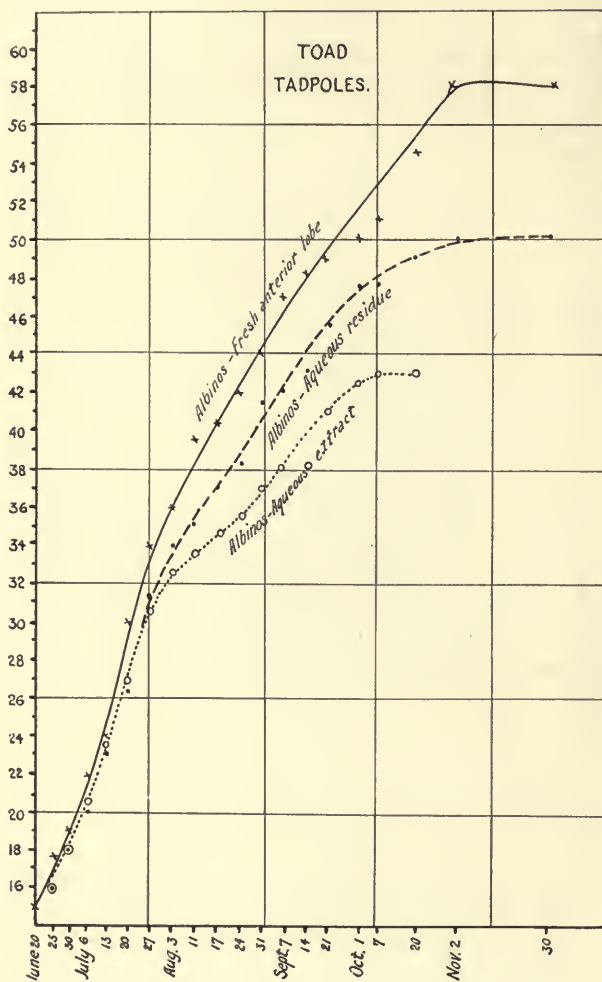


Fig. 8 Growth curves of hypophysectomized toad larvae supplied with fresh anterior-lobe substance, with aqueous extract, and aqueous residue of the anterior lobe. The ordinates represent the total length of the larvae expressed in millimeters.

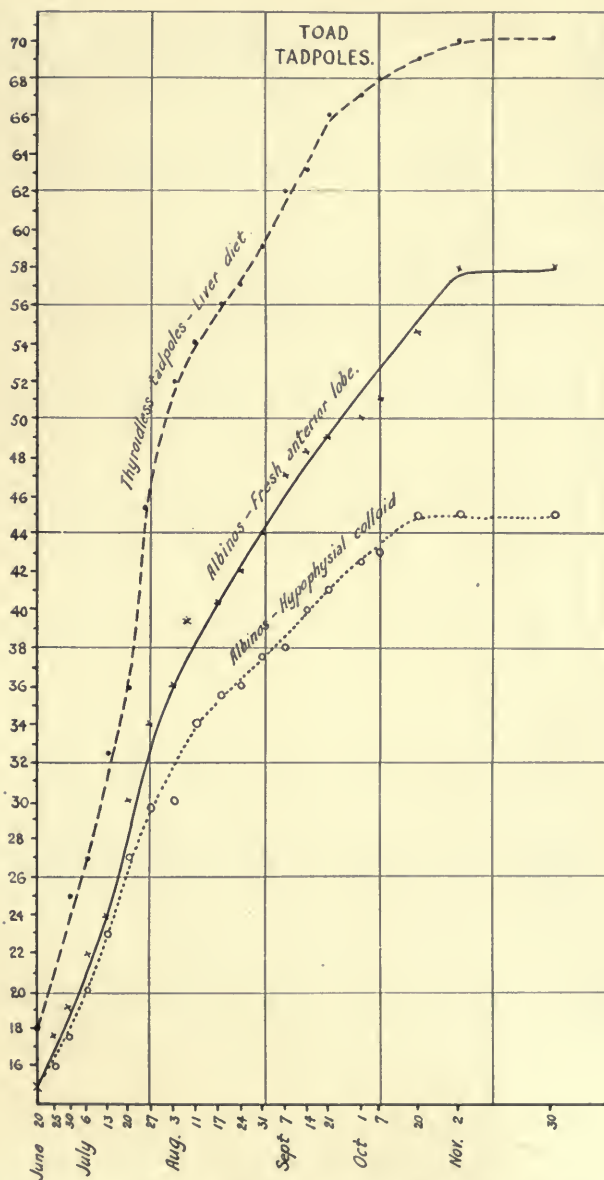


Fig. 9 Growth curves of thyroidectomized toad larvae supplied with liver, hypophysectomized toad larvae supplied with fresh anterior-lobe substance and with hypophyseal colloid. The ordinates represent the total length of the larvae expressed in millimeters.

TABLE 4

Table showing the marginal, minimal, and average lengths (most commonly occurring dimension) of the toad larvae on which the accompanying growth-curves are based

BUCCAL COMPONENT OF HYPOPHYSIS ABLATED										THYROID ABLATED	NORMAL
	Liver diet	DIET OF GLANDULAR LOBE OF HYPOPHYSIS						Liver diet	Fresh glandular lobe		
Date		Fresh glandular lobe	Alcoholic extract	Alcoholic residue	Aqueous extract	Aqueous residue	Colloid				
June 20.....	13-16 15	13-16 15	13-16 15	13-16 15	13-16 15	13-16 15	13-16 15	13-20 18	13-16 15		
25 ¹	15-17 16	15-20 18	15-17 16	15-17 16	15-17 16	15-17 16	15-17 16		15-17 16		
30.....	17-20 18.5	16-21 19	16-19 17.5	19-20 19.5	17-20 18	17-19 18	16-19 17.5	21-26 25	20-23 21.5		
July 6.....	17-21 19.5	17-23 22	18-22 20.5	20-21 20.5	19-22 20.5	19-21 20	19-21 20	21-29 27	22-26 24.5		
13.....	19-24 22.5	21-28 24	20-24 22	24-29 25	23-25 23.5	22-25 23	21-25 23	26-35 32.5	26-33 29.5		
20.....	21-29 26	23-33 30	24-28 25	26-30 28	25-32 27	24-29 26	24-29 27	29-42 36	30-37 33		
27.....	25-34 29	27-36 34	27-31 28	26-34 33	27-35 30.5	27-34 31	27-34 29.5	29-55 44	36-42 39		
Aug. 3.....	28-37 34	32-40 36	28-33 29.5	30-39 36	31-36 32.5	27-38 34	28-34 30	36-55 52	41-45 40		

Aug. 11.....	30-39 35	32-41 39.5	31-35 31.5	30-40 38	31-37 33.5	28-41 35	30-39 34	41-58 54	42-47 44
17.....	32-42 36.5	31-45 40	32-36 33.5	38-40 38	31-38 34.5	30-41 37	31-39 35.5	43-59 56	43-49 45
24.....	34-44 36.5	34-47 42	33-37 34.5	38-42 40	33-38 35.5	31-42 38	31-40 36	47-63 57	46-49 47.5
31.....	34-45 37.5	34-49 44	33-38 36	38-44 41	35-39 37	42-47 41.5	31-40 37.5	49-65 59	48-53 49
Sept. 7.....	37-46 39	37-53 47	35-40 36.5	40-46 43	36-40 38	42-48 42	31-42 38	49-65 62	47-57 50
14.....	38-45 40	39-54 48.5	35-39 37	40-46 44.5	35-41 38	42-49 43	31-43 40	52-72 63	Trans- formed
21.....	37-46 40	38-56 49	35-38 37	44-48 47.5	37-42 40	42-48 45.5	31-47 41	52-73 66	
Oct. 1.....	38-46 41	40-57 50	35-39 38.5	47-51 49	40-44 42.5	42-48 47.5	32-48 42.5	52-79 67	
7.....	38-45 41.5	40-60 51	36-39 38	48-52 50	40-46 43	42-49 47.5	32-48 43	55-77 68	
20.....	38-48 42.5	44-63 54.5	37-41 40	50-53 51	42-46 43	42-50 49	33-48 45	60-78 69	
Nov. 2.....	39-48 44	48-66 58	37-42 40.5	50-55 52	Accident- ally killed	42-52 50	35-48 45	64-80 70	
30.....	39-48 44	47-65 58	39-43 41	50-55 52		42-53 50	35-48 45	64-80 70	

¹ First date of special feeding.

effect has been produced in normal larvae by such alimentary régimes.

One article of diet, liver—the chief nutritive substance supplied—is common to the specimens of the second division. That the retardation in the growth rate of these specimens is not due to a growth-retarding substance in the liver, but to the absence of the hypophysial growth-maintaining substance, is clearly indicated by three lines of evidence: 1) we should have

TABLE 5

Table giving the number of normal, thyroidectomized, and albinous toad larvae on which the accompanying growth-curves are based

BUCCAL COMPONENT OF HYPOPHYSIS ABLATED								THYROID ABLATED	NORMAL
	Liver diet	DIET OF GLANDULAR LOBE OF HYPOPHYSIS						Liver diet	Glandular lobe diet
		Fresh glandular lobe	Alcoholic extract	Alcoholic residue	Aqueous extract	Aqueous residue	Colloid		
June 25	8	30	8	8	8	8	8	14	18
Aug. 13	7	23	7	8	6	8	8	13	18
Sept. 14	7	21	7	6	6	8	7	13	0
Oct. 20	6	20	4	6	0 ¹	6	7	8	0

¹ Killed accidentally.

to likewise assume that such growth-retarding substances were in adrenal cortex, adrenal medulla, and posterior lobe, for the same growth delay exists when albinous larvae are fed with any of these substances; 2) Mendel and Osborne ('18) have shown that the proteins of liver are adequate for the needs of nutrition in mammalian growth; 3) normal frog larvae supplied with a liver diet exhibit an entirely normal rate of growth.

It seems clear, then, that the normal growth rate of the albinous specimens receiving fresh anterior lobe or its residues is due to an alimentary replacement of the growth-maintaining

substance normally furnished by the tadpole's own gland. It is significant of the stability of this substance that its efficacy or physiological nature has not been seriously impaired by the severe treatment accorded it in the prolonged extraction with boiling water or absolute alcohol. It would seem certain that the retardation in the growth rate of the albinos receiving liver, hypophysial extracts, and colloid is due to the absence of the morphogenic principle normally furnished by the anterior lobe of the pituitary.

The growth rate of thyroidectomized larvae

It has been noted by previous workers (Allen, Hoskins) that thyroidectomized larvae attained a size much in excess of the normal and that the pituitary glands of these specimens were relatively increased in size. Since the larval condition persists in the thyroidless larvae and since the hypophysis is hypertrophied, it might be expected that these specimens would exhibit a more prolonged, and a more rapid rate of growth than even the anterior-lobe-fed albinous tadpole. By thyroidectomy, then, there would be obtained tadpoles which would simulate the rapid growth exhibited at times by the acromegalous human subject.

Observations, however, made on two groups of thyroidectomized frog larvae, one group (seventy-two specimens) fed on anterior lobe, the other (fifty-nine specimens) fed on liver, show that their growth is identical in rate with that of their normal brothers or with the albinos supplied with a continuous diet of the fresh anterior lobe (fig. 5). Their large size is never attained by a more rapid growth rate, but rather, as in the anterior-lobe-fed albinos, by a prolongation of the growth period. No variation in growth rate was caused by feeding the fresh anterior lobe of the pituitary, a non-effect already reported upon by Allen ('18). The enlarged pituitary which these animals possess does not appear to have supplied any growth stimulus in excess of the normal.

Nevertheless, a different condition obtains with the thyroidectomized toad larvae, at least when supplied with a liver diet.

This is well shown by the members of one group (fourteen specimens) whose growth rate was more rapid than that of their normal fellows supplied with anterior lobe substance (fig. 9). Moreover, they attained a size notably in excess of that reached by their hypophysis-fed albinous mates.

Evidently, then, in the case of the frog larvae the enlarged hypophysis, which Allen and Hoskins have shown to result from thyroidectomy, has not supplied any growth stimulus in excess of the normal. To what the acceleration in the toad larvae is due has as yet been undetermined, but that an increased secretion from the hypertrophied pituitary may be an important factor, as suggested by Hoskins, seems not improbable.

4. MODIFICATIONS IN THE SIZE AND STRUCTURE OF THE ENDOCRINE ORGANS

It seems probable that a tissue, whether its distribution be diffuse or localized, is more labile and 'adaptive' in its early formative stage than in its mature condition. An example of this is seen in the nervous system, which in its mature, highly differentiated condition loses its capacity for regeneration and 'adaptation,' a capacity which it exhibits to a surprising degree, however, in its early formative stages (Lewis), more especially when associated tissues can elicit a developmental response (Burr). It is likely, then, that if a disturbance be experienced by the endocrine system in its early and embryonic stages, greater alterations in its members will result than if this upset had been suffered later in the life of the individual. As has been previously suggested, the more extensive structural changes which are manifested by the internal secretory organs of the hypophysis-free tadpole as compared to the hypophysectomized mammal are probably referable to the earlier institution of this disturbance. Extensive structural modifications, as we will show, are invariably manifested in the thyroid, the neural portion of the hypophysis, and the interrenal and chromaffin components of the adrenal gland subsequent to the early loss of the epithelial hypophysis, while lesser though distinct changes are shown by the epithelial bodies. The behavior of the fat-organ in hypophysial deficiency is also described in this section.

The hypophysial components

The endocrine disturbance leading to the abnormal condition of the epithelial bodies, the thyroid and adrenal glands, subsequently to be described, has of necessity been mediated to these distant organs by the blood stream. The neural hypophysis, while subjected as are these other glands to this general endocrine disturbance, has, in addition, been subjected to the loss of its intimate anatomical associate, the epithelial hypophysis, a loss which in itself might conceivably greatly modify the neural lobe entirely apart from the general disturbance introduced into this correlative system of organs by buccal hypophysectomy. That the intimate anatomical association existing between the neural and epithelial components, to which attention has been called by many observers,³² is fundamental for the elaboration of the secretion of at least the posterior lobe, finds support not only in the anatomical evidence afforded by this relationship, but also in the recent work of Schmidt and May from the chemical side.³³ As will be pointed out, corroborative evidence bearing upon the significance of this coalescence will accrue from this study. It must be kept in mind that in this experimental work the buccal constituent of this gland has been removed prior to the union with its neural associate, which consequently remains undisturbed, and that the infundibular process at this time gives no indication of the structural differentiation which it is to later undergo, so that any modifications exhibited by the neural lobe (or the infundibular process) are not referable to any direct operative injury inflicted upon this member. As will be subsequently pointed out more in detail, sections and models of these albinos reveal a profound reduction in size, as well as an atypical shape and

³² Herring, Trautmann, Stumpf, Kohn, Erdheim and Stumme, Vogel, Stendel, and others.

³³ Schmidt and May, by appropriate chemical treatment of the alcoholic hypophysial extract, Tethelin, have secured a substance whose action simulates the active principle of the posterior lobe, *i.e.*, causes a contraction of the isolated uterus and produces a vasoconstriction.

abnormal position of the neural lobe.³⁴ Indeed, this malformation is not limited to the neural lobe, but is participated in also by the floor of the infundibular process. This floor, normally of considerable thickness, is membranous and folded in the albinous larvae.³⁵

It is of considerable importance, in view of the conditions obtaining in the albino, to have clearly in mind the parts of the infundibular process together with their structure in the normal animal, and more especially that of the apical and ventral portions. The walls of this process have three divisions:³⁶ 1) a ventromedial portion in contact with the pituitary gland, the 'pituitary' wall or floor; 2) a dorsal portion, the 'saccular' wall; and, 3) the lateral extensions of both these walls which form the shallow lateral concavities, the 'lateral processes.' The neural lobe in its early stage develops near the apex of the infundibular process, and in its fully formed condition is readily identified as a distinct element attached by a broad surface to the dorsal wall of the infundibular process, near its ventral border (fig. 25). An examination of the three portions of the infundibular process of the normal animal reveals the fact that these regions are of two structural types. One type, that exhibited by the lateral processes and saccular wall, is membranous and composed of a squamous ependyma with little or no ectally placed neuropile. The other type, that forming the pituitary wall, is thickened and composed of a high columnar

³⁴ The reduction in the size of the neural lobe in the albino was reported in The Anatomical Record. In the present paper more comprehensive data have been given.

³⁵ The hypophysis of an anuran larva, in common with other vertebrates, consists of four components, three arising from oral ectoderm, one from the infundibular process. The development of those lobes derived from the buccal rudiment (the pars intermedia, the pars glandularis and its paired cephalic processes, the pars tuberalis) conforms in general to that recently described by Atwell for *R. pipiens*. Wax models, $\times 133$, of the pituitaries of nine normal larvae, from 17 mm. in length to the young adult stage have been made (table 6). In addition to these, the neural lobes of ten albinos, ranging in size from 16 to 51 mm., and the neural and buccal lobes of nine partial albinos were modeled. The lobes of the pituitary are readily identified and their relationship to each other is constant (fig. 25, *a, b, c*), compare to Atwell's figure 13, page 81, but considerable variation in their relative size is shown.

³⁶ The terminology is essentially that of Tilney ('15).

ependyma with an ectally placed prominent stratum of neuro-pilem (fig. 28). In the albino the saccular wall and lateral processes and pituitary wall all exhibit the membranous type of structure (fig. 29). The pituitary floor, which is normally in contact with the epithelial hypophysis and which has been deprived of such contact by the epithelial hypophysectomy, thus exhibits the same type of structure as those portions not normally in contact with this epithelial component of the hypophysis. Directly attributable to the membranous character of this pituitary floor are invariably seen certain pronounced but irregular folds which appear in this structure in the albino (fig. 26), but not in the normal animal (fig. 25). Although such foldings are undoubtedly due to the technical procedure incident to the preparation of this material, yet the membranous nature of the infundibular floor of the albino is directly conducive to them, while the firmer structure of the normal infundibulum prevents them.

The abnormality in the infundibular process in the albino is not limited to the pituitary floor, for it is very evident that the neural lobe in the albino as compared to the normal animal of corresponding dimensions (table 6) is also diminished in size, a diminution which varies from 40 per cent to 80 per cent and which transcends very distinctly the considerable variability which this lobe exhibits in both types of specimens.³⁷ Associated with this diminution in size is a profound alteration in the shape and position of this lobe. Instead of the symmetrical, transversely placed, dumb-bell-shaped lobe of the normal (fig. 25), this dwarfed lobe is oval, asymmetrical in shape and position, and has no great transverse extent (fig. 26). Normally attached somewhat dorsally to the apex of the infundibular process and thus abutting and being attached to its dorsal surface, in the albino it is constantly found grasping the apex of this process and extends invariably slightly onto the pituitary

³⁷ It does not appear from figures 28 and 29 that the neural lobe of the albino is smaller than in the normal. It must be kept in mind, however, that due to the difference in the shape of this lobe in the two types that a median sagittal section passes through its smallest dimension in the normal animal, and through its maximum sagittal diameter in the albino (figs. 25, 26).

surface (figs. 26, 29). This lobe is thus profoundly modified by the loss of its associate. It attains normal development neither as regards size, form, nor position.³⁸ In its response to buccal hypophysectomy the reduction of this gland thus aligns it with the thyroid and the adrenal cortex reduction. Indeed, in those animals suffering only a partial loss of the epithelial hypophysis, the thyroid and adrenal glands are usually not altered in development, but the neural hypophysis is invariably atypical.

In an earlier section of the paper we have referred to the type of tadpole in which the removal of the epithelial hypophysis was incomplete. Since these animals usually manifest definite pigmentary alterations, we have designated them 'partial albinos.'³⁹ Especial interest attaches to the examination of the endocrine system, more particularly its hypophysial components in this type, since we have here an intermediate condition between a complete deficiency of the epithelial hypophysis and a normal structure. A study of this form throws much additional light upon the interesting relationship obtaining between the neural and epithelial components of the pituitary as well as supplying evidence bearing upon the endocrine locus responsible for the pigmentary deficiency.

In the partial albinos no uniformity in shape is displayed by the vestige of the epithelial hypophysis. It is usually oval in outline and of a variable thickness (figs. 27, 30). Only occasionally do we find it displaying the normal division into glandu-

³⁸ The findings of Allen (Abstracts, Am. Assoc. Anatomists, 1917) are distinctly at variance with those reported here. This author states, "The pars nervosa of the hypophysis forms normally in tadpoles, both *Rana* and *Bufo*, from which the anlage of the anterior lobe has been removed." As I have previously pointed out, a median sagittal section of the infundibulum usually shows a neural hypophysis of normal or even enlarged proportions. By modeling this component, unmistakable evidence concerning its diminution in size and atypical form is secured, however.

³⁹ I called attention in 1916 (Proc. Am. Assoc. Anat.) to an albinous tadpole in which a leg development took place. At that time the minute buccal fragment displayed by this animal had not been noted. Allen, in 1918, called attention to the complete metamorphosis of certain toad and frog tadpoles with an imperfect hypophysis and displaying a definite pigmentary deficiency.

lar and intermediate components. The size⁴⁰ of this vestigial gland is always considerably less than that obtaining in the normal tadpole of corresponding dimensions (table 6). As would be anticipated, this epithelial gland, subjected as it was to a varying surgical interference, leaves a vestige which shows great diversity in its position as well as in shape and size. That the position which this lobe assumes is of paramount importance in the influence it may exert upon the other glands and upon metamorphosis, is evident from a correlation of the structural changes enjoyed by the partial albino with the position which the buccal fragment has assumed. In the specimens thus far studied, three positions have been assumed by this fragment:

- 1) In the first case, this fragment, not separable into glandular and intermediate components, does not reach the caudal extremity of the infundibular process, nor does it touch at any point the true neural lobe (figs. 27, 30). The members of this group, which includes the partial albinos (p 1, p 2, p 3, p 4, p 5), all displayed a pronounced albinism. It is to be noted that none of these animals complete metamorphosis, though, as in the other groups, their thyroid glands are not atrophic.
- 2) In the second case, the fragment, not separable into glandular and intermediate components, does not reach its usual caudal position, but nevertheless attains a definite though small contact with the true neural lobe. This class includes a smaller number of specimens (p 7, p 9, p 10), the members of which display a variable pigmentary disturbance. They complete metamorphosis, though tardily, and the thyroid glands exhibit a pronounced colloidal hypertrophy (colloid goiter).
- 3) In the third case, the epithelial fragment, which is separable into intermediate and glandular components, assumes the position typical of the normal animal. One specimen only fell in this class (p 8); it did not exhibit a pronounced pigmentary upset; it completed metamorphosis and had a normal thyroid gland. A study of a

⁴⁰ The metamorphic changes in all the partial albinos, except p 1, which died from infection, whose hypophyses were modeled (table 6), had ceased for some time prior to the fixation of the animal. It is certain that the metamorphosis would never have been completed nor even materially advanced had the specimens survived longer.

TABLE 6
Weights of the wax models ($\times 133$) of the various lobes of the hypophysis

Individual specimen number	Type	SPECIMEN		WEIGHT OF THE MODELS IN GRAMS					
		Length ¹ in mm.	Age, dated from operative stage (days)	Diet	Pars neuralls	Pars inter-media	Pars glandularis and pars tuberalis	Total weight of pars epithelialis	No. of times heavier than pars neuralls
9	Normal frog tadpole	17 (total length)	29	Liver	0.18	Not separable		1.63	9.0
11	Normal frog tadpole	25 (total length)	39	Liver	0.43	1.03	1.48	2.51	6.0
13	Normal frog tadpole	38 -/-/3.0	60	Liver	2.55	5.04	10.72	15.76	6.1
15	Normal frog tadpole	38 -/-/4.5	63	Liver	2.97	4.47	9.33	13.80	4.6
17	Normal frog tadpole	43 -/-/6.0	101	Liver	1.88	3.11	5.16	8.27	4.4
19	Normal frog tadpole	43 -/-/9.0	101	Liver	2.66	4.60	5.20	9.80	3.3 [●]
21	Normal frog tadpole	50 17/33/15	111	Anterior lobe	4.03	8.51	8.01	16.52	4.1
23	Normal frog tadpole	47 17/30/14	124	Liver	7.07	15.46	10.83	26.29	3.7

¹ The length of the component parts of the tadpole is given in the following order: Total Body/Tail/legs /Hind/Fore legs

33	Normal frog tadpole	18 young 16/2 adult	356	Liver	5.94	7.12	4.61	11.73	1.96
35	Normal toad tadpole	19 young 17/2 adult	84	Liver	2.44	3.61	5.36	8.97	3.67
10	Albino frog tadpole	16.5 (total length)	29	Liver	0.12	-	-	-	-
12	Albino frog tadpole	23.5 (total length)	39	Liver	0.17	-	-	-	-
14	Albino frog tadpole	36 -/-/0.1	64	Liver	0.36	-	-	-	-
16	Albino frog tadpole	43 -/-/0.1	101	Liver	1.13	-	-	-	-
18	Albino frog tadpole	43 -/-/0.1	101	Liver	0.92	-	-	-	-
22	Albino frog tadpole	48 18/30/0.1	134	Adrenal cortex	0.79	-	-	-	-
24	Albino frog tadpole	51 -/-/0.3	191	Liver	1.51	-	-	-	-
26	Albino frog tadpole	54 -/-/0.3	191	Anterior lobe	2.60	-	-	-	-
28	Albino frog tadpole	66 22/44/1.5	197	Anterior lobe	2.61	-	-	-	-

30	Albino frog tadpole	19 13/6/2.0	122	Anterior lobe and thyroxin	-	-	-	-
p 1	Frog partial albino	36 ² 15/21/4.2	66	Liver	-	0.93	1.60	1.7
p 2	Frog partial albino	45 18/27/6.5	148	Liver	-	Not recog- nizable	2.24	-
p 3	Frog partial albino	43.5 16.5/27/6.0	243	Liver	-	1.38	1.47	1.09
p 4	Frog partial albino	46 18/28/20	221	Adrenal cortex	-	Not recog- nizable	4.98	-
p 5	Frog partial albino	39 17/22/15/7	218	Liver	-	5.67	4.53	0.8
p 7 ³	Frog partial albino	18 young 14/4 adult	356	Liver and anterior lobe	-	1.28	4.30	0.69
p 8 ²	Toad partial albino	16 young 16/- adult	144	Liver	1.36	1.60	4.14	2.8
p 9 ²	Toad partial albino	16 young 14.5/1.5 adult	144	Liver	-	1.95	1.05	0.5
p 10 ³	Toad partial albino	17 young 17/- adult	335	Liver	-	3.68	2.27	0.6

² Albinism scarcely perceptible. ³ Albinism pronounced.

greater number of 'partial' albinos, it is anticipated, will reveal an overlapping of these classes, but because of the apparent association between the progress of metamorphosis, the reaction of the thyroid gland and the position of this epithelial fragment, such a tentative classification appears justifiable and will be discussed further when the thyroid glands of these specimens are more specifically considered.

The description of the picture presented by the epithelial hypophysis of the partial albino would be incomplete unless we added a word in regard to the structural characteristic of this vestige. Its structure (in the first and second classes mentioned above) is characteristic neither of the glandular nor the intermediate components of the pituitary. Certain portions are composed of acidophilic cells, other portions of cells similar to those normally found in the pars intermedia. The organization of those cells, however, is typical of the pars intermedia, since the component cells are neither arranged in definite cords nor separated by numerous nor large sinusoids (fig. 30).

The response of the neural lobe to partial hypophysectomy is of even greater value in analyzing the interrelationship existing between the neural and epithelial components of the pituitary than was the reaction of this lobe to the complete loss of the buccal component, as obtains in the albino. In size it is usually diminished as compared to the normal animal of corresponding dimensions and stage of advancement (table 6) and at times the lobe as such is not even recognizable (specimens p 2, p 4). When present it is asymmetrical in shape and position and frequently encroaches upon the pituitary floor. Of great interest are the conditions obtaining immediately about the epithelial fragment. About this fragment there is formed a thickening of the neural tissue which we might designate as a 'novel' neural lobe. This partially surrounds and grasps the buccal fragment (fig. 30), an appearance which in part may be explained by the indentation which this lobe makes in the brain substance. When the usual neural lobe is absent or greatly diminished in size (specimens p 2, p 4), there is evident in those specimens that have been thus far examined a thickening of the pituitary floor

(fig. 30), a condition which strongly suggests that the neural lobe had migrated cephalically and was attempting to assume a juxtaposition with the unusually placed epithelial fragment. This seems the more probable, since in the specimens which have suffered a complete loss of the epithelial hypophysis there is always a readily distinguishable neural lobe. That the epithelial hypophysis apparently exhibits not only this 'attractive' force upon the neural lobe, but definitely stimulates the growth of adjacent neural tissue, is shown both by the thickening of the neural tissue about the epithelial hypophysial vestige⁴¹ in the partial albinos and by the negative evidence supplied by the absence of the usual thickening of the pituitary floor and diminished size of the neural lobe in the albino which displays no epithelial hypophysis whatever. It thus seems clear that in the absence of a nearly normal epithelial component the neural hypophysis cannot undergo its normal development nor attain its typical shape or size.⁴² That the neural lobe can and does form even in the absence of its associate, however, is clear, and it seems equally clear that in those specimens in which a sufficient metamorphic stimulus is supplied, whether by the thyroid or the hypophysis or jointly, that this lobe can attain a very considerable size.⁴³

⁴¹ Although this neural lobe simulates in structure the typical lobe, nevertheless, it seems doubtful whether it is able to assume the functions of that lobe. That it can assume this function appears improbable both from the failure of such specimens to metamorphose as well as from the serious pigmentary disturbance which they display.

⁴² To what an extent the general disturbance in this correlative system of organs plays a part is problematical. It is difficult to determine, save by grafts of the buccal component with the hypophysectomized larvae, an undertaking which has not been fruitful in the hands of the author. A similar failure to secure significant effects has recently been reported by the Hoskinses. Likewise, efforts to secure an apposition of the buccal component and the lateral or dorsal walls of the infundibular process have so far failed. The author hoped to determine by such misplacement of the buccal component whether these walls would undergo the hypertrophy normally exhibited by the pituitary wall.

⁴³ No clear evidence is at hand showing whether the buccal lobe would undergo a normal development in the absence of its neural associate. Suggestive evidence, however, that it can develop independently is furnished by the work of Habermeldt ('09). According to this author, the pharyngeal hypophysis, although far remote from any neural associate, parallels in development and differentiation the pars glandularis of the hypophysis.

A study of the older albinous and partially hypophysectomized larvae does not throw light upon the developmental process which has led to the thinning of the pituitary floor and the diminution in the size of the neural lobe in these specimens. From such a study we can conceive that one of two developmental processes lead to this anatomical condition. In the first place, it is possible that a normal differentiation and development of these parts may have taken place, so that at some not very early period they were structurally identical in both the albinous and normal larvae. Later, due to the absence of the association with the epithelial hypophysis, these normally developed parts underwent an atrophy which led to the conditions just described. Or, in the second place, it is possible that, due to the absence of the 'stimulatory' force normally supplied by the epithelial hypophysis, the neural lobe and pituitary floor even from their earliest stages did not undergo the normal increase in size. A developmental study of the albino, the normal, and the partially hypophysectomized tadpole affords clear evidence that the second hypothesis is correct. An examination of the divisions (lateral, saccular, and pituitary) of the infundibular process in an early stage (5 to 10 mm.) reveals the fact that these parts are nearly identical in structure and that no differences exist between a normal larva and a larva which has suffered the loss of its epithelial hypophysis. This infundibular pocket, slightly thinner at its apex where it is formed of a cuboidal epithelium, thickens near its attachment to the firmer walls of the brain where it is formed of columnar epithelium. As development proceeds, the dorsal and lateral walls of this pouch become progressively thinner in both the albino and normal animal, and at a 14- to 16-mm. stage their epithelium is of the squamous type and remains so through their larval life span (fig. 10). Thus the development of the dorsal and lateral walls (save the small portion giving rise to the neural lobe) is identical in the two types of animals. It is in the development of the pituitary wall, however, that the differences between the albinous and normal larvae make their appearance. This portion, both in the albino and the normal, undergoes for

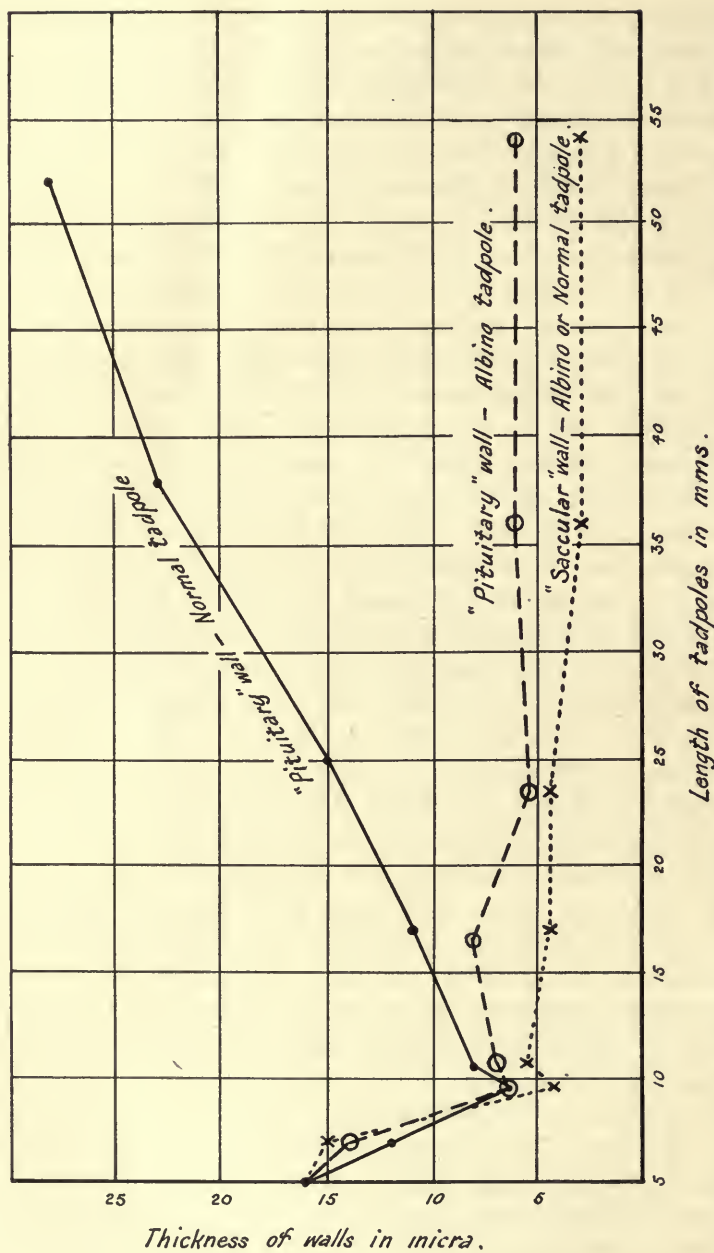


Fig. 10 Curves showing the changes in the thickness of the infundibular process in normal and albinous frog larvae during development.

a time the same thinning as do the dorsal and lateral walls. By a 9.5-mm. stage the pituitary wall has become much thinned, and consequently the various portions of the infundibular process are approximately of the same thickness (fig. 10) and nearly structurally identical. From this point a series of changes take place leading to a very different condition in the pituitary wall of the two types. In the albino the membranous wall persists. In the normal this becomes progressively thicker and finally attains a thickness many times that obtaining in the 9.5-mm. stage. It is significant that the epithelial hypophysis, although previously in contact with a small portion of the infundibular floor prior to a 10-mm. stage, institutes an intimate juxtaposition with the pituitary wall of the infundibular process at the time when the latter loses its membranous character and begins to thicken.

Differences between the neural lobe of the albino and the normal appear at a later stage than they do in the pituitary wall. The thickening near the apex of the infundibular process indicating the first formation of the neural lobe makes its appearance at approximately a 14-mm. stage in both the normal and albinous tadpole,⁴⁴ and is nearly identical in position and size in both types at a 16- to 17-mm. stage. From this stage the neural lobe in the normal increases concomitantly with the growth and lateral extensions of the developing pars intermedia. In the albino, on the contrary, a slower growth (table 6) and relatively slight lateral extension take place—a process leading to the small, malformed neural lobe of the albino.

The small neural lobe and the membranous pituitary wall of the albino are thus clearly referable to a non-development rather than to an atrophy of a normally sized structure. The walls of the early infundibular process have apparently two inherent possibilities after having undergone the primary thinning: one, to retain this membranous characteristic as do both the dorsal and lateral walls of the normal or albinous larvae and the pituitary wall of the albino; the other, to develop into

⁴⁴ According to Atwell, this lobe becomes recognizable in an 18 to 20 mm. stage in *R. pipiens*.

the thickened wall characteristic of the pituitary floor of the normal specimens. If freed from the influence of its buccal associate and left to its own independent development, the membranous structure persists; under the stimulatory influence of its buccal associate, a thickening results, leading to the solid, firm structure of the normal tadpole. Curiously, in the absence of the buccal hypophysis, the inherent capacity for growth expresses itself in the neural lobe as if an hereditary influence here exerts itself and leads to the formation of a 'vestigial' structure. That the epithelial hypophysis does exert a stimulatory effect upon the adjacent neural tissue seems clear, as has been pointed out, not only from the evidence accruing from a study of the albino in its different stages, but from the 'partial' albino as well. The diminutive and misplaced fragment of the buccal hypophysis in these specimens has invariably formed about itself a brain lobe, identical in structure, so far as can be determined, with the normally placed pars nervosa of the pituitary. Moreover, the 'influence' of this vestigial epithelial gland appears to be effective at some distance, as indicated by certain specimens which, in sharp contrast to the albino, have no apically placed neural lobe. This condition suggests that this lobe has either been inhibited and a new atypically placed lobe formed *de novo* or it has been attracted to a new position by this misplaced epithelial fragment.

The inherent capacity for growth existing in the three portions of the infundibular process and the response of these portions to the presence or absence of the buccal component of the hypophysis can perhaps best be shown by schematic curves illustrating the reaction of the pituitary floor and one of the other portions of the infundibular process under the two conditions of normality and buccal hypophysectomy (fig. 10). These curves show the development of the pituitary wall in a normal tadpole and its albinous mate, together with a third curve showing the development of the dorsal and lateral walls of the infundibular process in either normal or albinous tadpoles.

Early in this section of the paper we stated that two lines of evidence indicated that the neural lobe is dependent upon its epithelial companion for its complete development. These briefly are: 1) certain cellular elements from the pars epithelialis (pars intermedia) apparently migrate into the neural component of the hypophysis and contribute to its secretion; 2) by appropriate chemical treatment a substance can be secured from the anterior lobe which displays certain of the physiological characteristics typical of extracts of the posterior lobe of the pituitary. From this study a third line of evidence from the experimental and developmental work herein detailed has been secured; 3) the neural lobe and pituitary floor are dependent upon the association with the epithelial hypophysis for their full development. The epithelial hypophysis then appears to exert a stimulating effect upon these structures and even upon any adjacent neural tissue, as is shown by the hypertrophy of the neural tissue about the atypically placed epithelial lobe.

The thyroid

Sections of the larger albinous larvae show that the thyroid glands of these specimens have suffered a profound atrophy, an atrophy so intense that the remaining vestige could hardly be suspected of playing any important physiological rôle. That, in fact, this is the case has recently been shown by Allen, whose experimental animals, suffering a double extirpation of the thyroid and buccal portion of the hypophysis, do not differ from those deprived only of the epithelial hypophysis. Indeed, the interrelationship obtaining between the hypophysis and thyroid appears to be of such an intimate nature that the ablation of the former, even in the midlarval stages, effects an atrophy of the latter (Adler, '14).

The thyroid gland, then, is greatly diminished in size (table 7, figs. 31 to 34) and modified in structure in those tadpoles suffering from an early and complete loss of the epithelial hypophysis. By this atrophy the thyroid is diminished, in tadpoles in excess of 36 mm. total length, to one-sixth or less of its normal size.

TABLE 7
Weights of wax models ($\times 133$) of thyroid gland

Individual specimen number	SPECIMEN				WEIGHT OF MODELS IN GRAMS		
	Type	Length ¹ in mm.	Age, dated from operative stage (days)	Diet	Right lobe of thyroid	Left lobe of thyroid	
7	Normal frog tadpole	10 (total length)	15	Liver	0.44	0.42	
9	Normal frog tadpole	17 (total length)	29	Liver	0.82	0.81	
11	Normal frog tadpole	25 (total length)	39	Liver	1.20	1.10	
15	Normal frog tadpole	38 -/-/4.5	63	Liver	18.72	17.94	
23	Normal frog tadpole	47 17/30/14	124	Liver	29.01	36.96	
21	Normal frog tadpole	50 17/33/15	111	Liver	29.45	42.18	

¹ The length of the component parts of the tadpole is given in the following order: Total /Hind/Fore Body/Tail/legs /legs

33	Normal frog tadpole	16 18/2	young adult	356	Liver	62.78	74.22
25	Normal frog tadpole	34 14/20/18/10		111	Posterior lobe	22.32	28.55
27 ²	Normal frog tadpole	10 10/-	young adult	111	Posterior lobe	19.36	25.36
35	Normal toad	19 17/2	young adult	84	Anterior lobe	14.53	14.3
8	Albino frog tadpole	10	(total length)	15	Liver	0.43	0.41
10	Albino frog tadpole	16.5	(total length)	29	Liver	0.41	0.43
12	Albino frog tadpole	23.5	(total length)	39	Liver	0.41	0.42
14	Albino frog tadpole	36 -/-/0.1		64	Liver	2.05	2.21
16	Albino frog tadpole	43 -/-/0.1		101	Liver	3.07	3.11

² This specimen suffered considerable shrinkage during the technical manipulations, consequently the thyroids are probably somewhat diminished in size by this shrinkage.

TABLE 7--Continued

SPECIMENS				WEIGHT OF MODELS IN GRAMS		
Individual specimen number	Type	Length: in mm.	Age, date from operative stage (days)	Diet	Right lobe of thyroid	Left lobe of thyroid
18	Albino frog tadpole	43 -/-/0.1	101	Liver	2.80	2.57
24	Albino frog tadpole	51 -/-/0.3	191	Liver	3.75	2.57
30	Albino frog tadpole	19 13/6/2	122	Liver and thyroxin	3.82	5.88
p 1	Frog partial albino	36 15/21/4.2	66	Liver	15.87	16.60
p 4	Frog partial albino	46 18/28/20	221	Adrenal cortex	28.95	26.72 Accessory thyroid = 2.25
p 7	Frog partial albino	18 young 14/4 adult	356	Liver and anterior lobe	97.05	112.15
p 8	Toad partial albino	16 young 16/- adult	144	Liver	17.58	17.67
p 9	Toad partial albino	16 young 14.5/1.5 adult	144	Liver	44.98	51.52 Accessory thyroid I = 1.33; II = 1.42
p 10	Toad partial albino	17 young 17/- adult	335	Liver	55.52	56.12 Accessory thyroid = 6.74

Structurally, this atrophy is expressed by a reduction in the size of the follicles and in their colloid content (figs. 33 to 36). Indeed, certain cell clusters would not be recognizable as a follicular remnant, save for their location, so atypical are they. Other follicles exhibit the characteristic organization and may even contain an insignificant amount of poorly staining colloid. In no case do we see the large-sized, colloid-filled follicle of the normal animal. The component cells of the follicles appear also to have suffered from the hypophysial deprivation. Their cytoplasmic content is very noticeably diminished. This reduction in the size of the component follicles, together with an apparent disappearance of some of them, results in a loose organization of the gland, the majority of the follicles being separated by wide intervals. Had these interfollicular spaces been excluded in the models, an even greater discrepancy between the size of the thyroids of the albino as compared to the normal would have been shown.

Although not evident in specimens of 10 mm. total length (eighteen days after the operative stage), by the time larvae have reached a length of 17 to 18 mm. (twenty-nine days after the operative stage) the thyroid of the albinos is slightly but invariably smaller than that of the controls. This discrepancy progressively increases and is strikingly expressed in a 24-mm. (thirty-nine days after the operative stage) albino as shown in table 7. The pronounced atrophy of this gland in the albino is not due, then, to a regressive process in the normal gland; it is a phenomenon of retarded and atypical development.

It will be recalled that the 'partial' albinos, because of the position assumed by the atypical epithelial fragment of the hypophysis, were grouped into three classes: 1) those in which there was no contact between the true neural lobe and the epithelial fragment; 2) those in which there was a definite but slight juxtaposition between these two components; and, 3) those in which the epithelial fragment though diminished in size nevertheless displayed its usual division into glandular and intermediate lobes, which in turn displayed the usual relation with the infundibular derivatives. Curiously, the thyroid glands of

none of the animals included in these three classes are atrophic, though the epithelial hypophysis be but one-sixth of its normal size; indeed, those included in the second class exhibit greatly hypertrophied thyroids with the frequent formation of accessory glands (table 7, p 7, p 9, p 10). It will further be recalled that only the specimens of the second and third classes completed metamorphosis, while those of the first class underwent an abrupt and persistent metamorphic stasis at some stage in this process. It would thus appear that by the slight reaction between the true neural and the buccal components of the hypophysis, a sufficient 'hormonal' substance was supplied to cause an hypertrophic response of the thyroid gland.⁴⁵ These tadpoles metamorphosed (class 2, specimens p 7, p 9, p 10). On the other hand, if a larger amount of secretion was supplied by the pituitary (class 3, specimen p 8), no hypertrophy of the thyroid then resulted. A tentative explanation of the correlations obtaining between the epithelial and neural lobes of the hypophysis and the thyroid in the first class of animals, those in which no contact is made between the true neural lobe and the epithelial fragment, is more difficult. Since the thyroid is of normal size in these animals, it is evident that no compensatory response of this gland was evoked, although there would seem to be a need for such a response, since these animals did not complete metamorphosis. It would appear that some interaction between the true neural lobe and the epithelial lobe is necessary in order to evoke a hypertrophic response of the thyroid. Thus a functional specificity attends this union of true neural lobe tissue with the reduced epithelial component of the gland. The thyroid does not hypertrophy and metamorphosis appears never to be completed when the epithelial hypophysis comes in contact with only an atypical or a new neural lobe.

⁴⁵ It is of interest to note in this connection that the thyroids, which were subjected to microscopic examination, in Cushing's canine hypophysectomies, exhibited an excess of colloid. Further, that partial hypophysial extirpations in two human patients resulted in thyroid enlargement (Exner, cit. after Cushing).

The conditions then obtaining in these three classes of tadpoles seem to indicate that the epithelial fragment where separated from the true neural lobe, either in itself or through its reaction with brain tissue, other than the true neural lobe, is able to stimulate the thyroid to normal development, but that under these conditions metamorphosis cannot be completed; that when this fragment is in very slight contact with the neural lobe an hypertrophied thyroid results and complete metamorphosis is effected, and that when the usual relations of a completer contact between the two parts of the hypophysis obtain no hypertrophy of the thyroid occurs and we have the completion of the metamorphic processes. It might thus appear that the activity of the thyroid is thus the sole or determining factor on which the metamorphic processes are dependent. That such may not be the case seems clear from evidence gained by thyroid feeding. In thyroid and thyroxin feeding extending for periods in excess of six weeks and during which time daily minute doses of thyroid or thyroxin were administered, I have not been able to bring to complete metamorphosis a typical hypophysis-free albinous tadpole. The early stages in this process were passed through (leg growth, partial tail absorption), but the tadpole could not be carried past a certain stage, death invariably supervening. This appears to be the case also with the 'partial' albinos of class 1, whose metamorphic processes could not be carried to completion nor materially advanced after the onset of the metamorphic stasis by instituting a carefully controlled thyroid dosage. The results secured from the feeding of thyroid to completely and partially hypophysectomized tadpoles stand in sharp contrast to similar feeding experiments with the thyroidectomized tadpoles, which were readily completely metamorphosed by this treatment. It is not to be denied that a more carefully regulated or a different dosage of thyroid might bring about complete metamorphosis in the albino, but that it would do so appears improbable from the evidence at hand. The evidence thus indicates that metamorphosis is dependent upon the active principle of at least two glands; in the absence of either, the larval condition persists. One gland, the thyroid,

can apparently initiate the process, but unless there be some hypophysial secretion (and it would appear that this must be formed at least in part by the interaction of the true neural lobe with the epithelial component) the process apparently cannot be completed.

The adrenal cortex and medulla

If the abdomen of a normal tadpole in the later larval stages be opened and the ventral surface of the mesonephros be examined under the binocular, there will be seen a longitudinally placed whitish-appearing cord. This cord, irregular in outline, extends from a point somewhat cephalad to the kidney, over the anterior four-fifths of this organ (fig. 37). This is the adrenal gland.

When such a specimen is dropped into an alcoholic solution of sudan III or scarlet R, this cord takes on a reddish hue. Subsequent treatment with potassium bichromate reveals, however, that not all the cells of this column are tinted with the fat dye, for with chromation, groups of browned cells surrounded by the lipid-containing cords are revealed. Similar differential staining of the cortex is shown by osmium vapor or when an osmic-bichromate solution is used (figs. 38 to 41). If potassium bichromate alone is used, however, it can be readily seen that only the centrally placed cells of this strand give the chromaffin reaction, a reaction participated in also by certain groups of cells surrounding the central intestinal artery and neighboring parts of the aorta. By these staining methods we may thus clearly show the two components of the adrenal gland, the medulla exhibiting the chromaffin reaction and the cortex, or interrenal component, characterized by its lipid content. The arrangement of these two components resembles with surprising accuracy that of the adult as described by Stilling ('98) and Grynfeldt ('04).

The adrenal components of a thyroidectomized tadpole do not differ in any marked degree from those of the normal animal, yet there appears to be an increase in the cortical or interrenal tissue not entirely explained by the larger size reached by these animals (table 9).

Albinous tadpoles, on the contrary, reveal notable variations even on surface view from the picture presented by the two normally pigmented types (fig. 39). Although the kidney is of normal size, the adrenal column appears much more slender and more mesially placed than in a normal or thyroidectomized specimen of corresponding or even of inferior size. Not only is the column more delicate, but its caudal extent is somewhat less, its cephalic extent, however, corresponding to the normal. Sudan III does not color the column deeply, while the osmic-bichromate solution produces only a grayish coloration in contrast to the dark brown of the normal gland. Treatment with potassium bichromate gives an effect nearly identical with that of the normal. We thus have evidence, from surface views alone, which leads us to suspect that the adrenal cortex of the albino is diminished.

A section study of the three above-described types of animals lends corroborative evidence of an unquestioned nature to that furnished by the surface examination. For such a study it is essential that the technique employed not only preserve the lipid content of the cortical or interrenal cells, but that the medulla be clearly, although not necessarily differentially, stained. The most satisfactory fixing fluid has proved to be an osmic-bichromate mixture (Flemming's fluid less the acetic acid) which, although blackening the lipoids, does not interfere with the subsequent staining of the other tissues. Curiously, the chromaffin reaction is not shown when the osmium and bichromate, either mixed together or subsequent to each other, are used. Following fixation, immersion in 50 per cent. alcohol for twenty-four hours deepens the color of the lipid granules and appears to render them less soluble in the higher alcohols and the clearing agents. The sections (5 to 8 μ) are rapidly run down to 60 per cent. alcohol and then stained for two to three hours in Babes' safranin at 36°. After rinsing in alcohol, rapidly dehydrating and mounting, there is presented a preparation in which the lipid granules, a few of which have apparently been partially dissolved, are distinctly shown and in which the chromaffin cells can be unmistakably identified by their red tinge.

Further evidence that this identification is not incorrect was secured by fixation in Müller's fluid followed by a prolonged mordantage in potassium bichromate, the sections then showing the reticular fat-free cortical cells and the browned medulla.

By the employment of these two methods it can be readily determined that this longitudinal cell mass, aside from blood-vessels, connective tissue, and an occasional readily identified nephric tubule, is composed entirely of adrenal cortex and medulla.

The cortical cords, varying somewhat in width, are usually formed of three or four cell columns. They measure in the normal animal from 30 to 48 μ in diameter, an average of 36.6 μ . In the thyroidectomized specimens they are from 24 to 56 μ in diameter, an average of 36.5 μ (table 8). The individual cells composing these cords are of approximately the same size in the two animals, in the normal averaging 15.1 μ in diameter, in the thyroidectomized specimens 16.5 μ . These cells are composed of a mass of lipid granules imbedded in a cytotreticulum and surrounding a nucleus approximately centrally placed. This structure, then, after the osmic-bichromate solution gives a coarse, dark granular appearance, or after ordinary fixation and treatment with fat solvents reveals a delicate reticular cell.

If we compare these cortical cords, or cells, of the albino with those of the unoperated tadpole (figs. 38 to 41), we find that they are decreased in size in the former. The cords (in a 55-mm. albino) vary from 24 to 32 μ in diameter, an average of 23.7 μ , a reduction of approximately 33 per cent. from the normal. The cells vary from 12 to 16 μ in diameter and average 12.7 μ (table 8), a reduction of approximately 25 per cent. from the normal. It is difficult to determine, because of their profuse branching, whether the cords are actually reduced in number in the albino, but apparently this is the case.

The interrenal cells of the normal or thyroidless animal are browned by osmic; those of the albino assume a gray tone (figs. 38, 39). This would appear to be due to a different reaction of these granules and not to their volume, since the granules of both types of specimen are blackened by subsequent treatment with a low grade of alcohol.

TABLE 8

Table showing the variations and average widths of the interrenal cells and cords, together with variations and average area of the adrenal medullary cells (frog tadpoles)

SPECIMEN				DIAMETER OF CORTICAL CELLS IN μ				
Individual specimen number	Type	Length ¹	Age, dated from operative stage (days)	Diet	Minimal	Maximal	Average	Number of counts
37	Normal	39 15/24/4.5	187	Liver	13.6	18.4	16.1	8 •
t 1	Thyreoidectomized	55 19/36/1.0	187	Liver	13.6	22.4	17.5	14
32	Albino	54 19/35/0.2	187	Anterior lobe May 6 to July 18; then liver	9.6	16.0	12.5	11
37	See above	See above			Diameter of cortical cords 2 cells wide in μ			
t 1	See above	See above			32	44	36.6	8
32	See above	See above			32	49	36.5	15
					22	32	23.7	11
37	See above	See above			Area of single medullary cells in sq. cm.			
t 1	See above	See above			0.0005	0.0017	0.00091	18
32	See above	See above			0.00025	0.0010	0.00074	25
					0.0005	0.0019	0.0013	19

¹ The length of the component parts of the tadpole is given in the following order: Total Body/Tail/legs /Hind/Fore legs

In order to determine accurately the diminution in the adrenal cortex in the albino and its increase in the thyroidectomized tadpole, this substance was accurately drawn with the camera at a magnification of 300 diameters in three specimens, a 67-mm. albino, a 56-mm. thyroidless tadpole, and a 42-mm. normal animal. The drawings were transferred to a wax plate of proportionate thickness and the weight of the models of the cortex

TABLE 9

Table giving the weight of wax models ($\times 300$) of cortex of left adrenal (frog tadpoles)

SPECIMEN					Weight of model in grams
Individual specimen number	Type	Length ¹	Age, dated from operative stage (days)	Diet	
36	Albino	67 -/-/0.2	228	Anterior lobe May 6 to July 18; then liver	144.43
41	Normal	42 -/-/12	228	Liver	382.75
t 2	Thyroidectomized	56 -/-/2.0	228	Anterior lobe	759.60
p 11	Partial albino	41 -/-/16	286	Same as spec. 36	360.15

¹ The length of the component parts of the tadpole is given in the following order:
Total /Hind/Fore

Body/Tail/legs /legs

thus secured for these three specimens (table 9). The cortical substance of one adrenal—the left in each case—was treated in this way. The albino, although 11 mm. longer than the thyroidectomized tadpole, and 25 mm. longer than the normal specimen, had but 38 per cent. of the cortical tissue presented by the normal and 19 per cent. of that of the thyroidectomized specimen. It would thus appear to be established that the cortical tissue is greatly diminished in the albino as compared with

the normal or thyroidectomized tadpole. On the other hand, the cortical adrenal tissue of the thyroidectomized tadpole exceeded by twice that of the normal. Although the thyroidless animal is somewhat larger than the normal (11 mm.), yet the discrepancy in the size of the adrenals is out of proportion to the difference in the size of the specimens, and it thus seems certain that the cortical tissue is hypertrophied in the thyroidless larva as compared to the normal.

Changes as striking but of a different nature are evident between the adrenal medulla of a normal or thyroidectomized tadpole and a hypophysectomized tadpole. In the normally pigmented larva the medullary cells present a diverse appearance, since certain cells are deeply stained and the cytoplasm limited by a definite cell membrane, while the opposite extreme is seen in certain other cells whose cytoplasm is reticular or even vacuolated and but slightly tinged by the dye, the cell membrane not being distinguishable (fig. 40). Between these two extremes all gradations can be found, a condition suggestive of the various secretory states exhibited by the mammalian chromaffin tissue. In the albino, on the other hand, the cells are of one type; their cell boundaries are distinct and their cytoplasm moderately and uniformly stained (fig. 41). Moreover, the cells are uniformly larger than those either of the normal or thyroidectomized tadpole as shown by table 8.

Not only are the chromaffin cells larger in the albino, but their relation to each other and to the surrounding tissue is peculiar to these animals. They closely approximate each other and the adjacent cortical tissue, spaces between or around them being seldom evident. In the normal or thyroidectomized tadpole, on the other hand, an interval frequently separates these cells from each other and from the cortical tissue. This condition, which simulates shrinkage, was at first believed to be an artifact. Its constant occurrence in the normal and absence in the albino with identical technique suggests two possible causative factors. Either these spaces exist during life or the physical constitution of the cells of the normal and thyroidless larvae is such that shrinkage inevitably occurs during the manipu-

lations involved in the technical treatment. Be the explanation as it may, it is certain that in the preparations the chromaffin tissue of the normal animal almost invariably does not fill the intracortical space accorded it.

A duplication in wax of the volume of the medulla ($\times 187.5$) has been made for each of the three types of animal. In the normal and thyroidectomized animals the total intracortical medullary space has been included. It is evident, then, that if

TABLE 10
Table giving the weight of wax models ($\times 187.5$) of adrenal medulla (frog tadpoles)

Individual specimen number	SPECIMEN				Weight of model in grams
	Type	Length ¹	Age, dated from operative stage (days)	Diet	
34	Albino	54 19/35/0.2	217	Anterior lobe May 6 to July 18; then liver	27.32
39	Normal	39 15/24/4.5	217	Liver	14.93
t 3	Thyroidectomized	55 19/36/1.0	217	Anterior lobe	24.46

¹ The length of the component parts of the tadpole is given in the following order:

Total /Hind/Fore

Body/Tail/legs /legs

only the actual chromaffin tissue exclusive of the surrounding spaces had been drawn, an undertaking too laborious and uncertain to be considered, the mass of the model in the normal and thyroidless larvae would have been considerably reduced. From the included table it will be seen that the volume of the medulla while not seriously out of proportion to the size of the specimens would appear to be increased in both the albino and the thyroidless larvae (table 10).

Attention has already been repeatedly called to the incompletely hypophysectomized tadpole, the so-called 'partial' albino,

and their characteristics described. Especial interest is associated with these specimens in the study of the adrenal, since the pigmentation in Addison's disease is usually referred to a derangement of the adrenal. Both whole mounts and sections, however, reveal the fact that neither the adrenal cortex nor medulla suffers a serious disturbance in the 'partial' albino as compared with the normal animal. To test more exactly the amount of cortex in one such specimen, it was reproduced in wax ($\times 300$), as has been previously explained. These findings show that the cortical tissue is present in relatively as great an amount as in the normal tadpole (table 9). Apparently, then, as in the case of the thyroid, a relatively small amount of hypophysial tissue is sufficient to give rise to a normal adrenal.

The epithelial bodies

The epithelial bodies (Maurer) might be suspected of participating in the general endocrine upset experienced by the tadpole suffering from pituitary deficiency. Such, indeed, appears to be the case, although these bodies do not suffer as do the thyroids, adrenal cortex, or neural hypophysis. Models of these glands made from four normal and four albinous tadpoles reveal in many cases a profound diminution in the albino, although in other cases this decrease does not transcend the limits of variation of the individual bodies in the normal. When we take cognizance of the total amount of tissue, however, there can be no question but that it is profoundly diminished in the albino (table 11). There appears to be no serious structural abnormalities in these bodies. Thus in their reaction to epithelial hypophysectomy these bodies align themselves with all the other endocrine organs thus far examined (thyroids, adrenal cortex, neural hypophysis) save one, the adrenal medulla. An opposite response—one of increasing size—is evoked in the epithelial bodies (Allen) by thyroidectomy. In this opposed response in thyroidectomy they thus align themselves with the enlargement which the pituitary (Allen, Hoskins) and adrenal cortex (Smith) enjoy.

TABLE 11
The weights of the epithelial bodies modeled in wax ($\times 133$) of normal and albinous frog larvae

Individual specimen number	SPECIMEN			EPITHELIAL BODIES					
	Type	Length ¹	Age, dated from operative stage (days)	Diet	Right I	Right II	Left I	Left II	Total
17	Normal frog tadpole	43 -/-/6.0	101	Liver	grams Injured	grams Injured	grams	grams	grams
19	Normal frog tadpole	43 -/-/9.0	101	Liver	9.65	4.89	8.93	4.14	27.61
21	Normal frog tadpole	50 17/33/15	111	Anterior lobe	7.06	4.93	9.81	4.83	26.63
23	Normal frog tadpole	47 17/30/14	124	Liver	22.05	11.67	20.50	14.13	68.35
16	Albino frog tadpole	43 -/-/0.1	101	Liver	2.71	1.95	3.06	1.78	9.50
18	Albino frog tadpole	43 -/-/0.1	101	Liver	4.90	3.20	3.23	3.23	14.56
24	Albino frog tadpole	51 -/-/0.3	191	Liver	4.98	5.12	5.08	4.31	19.49
26	Albino frog tadpole	54 -/-/0.3	191	Anterior lobe	3.83	2.73	4.95	2.16	13.67

¹ The length of the component parts of the tadpole is given in the following order: Total /Hind/Fore Body/Tail/legs /legs

The fat-organ

Attention is called to the fat-organ, a structure which serves as a fat storehouse and as such is subject in the adult frog to seasonal and nutritional variations (Gaupp), since the behavior of this 'organ' in the tadpole suffering from hypophysial deficiency strongly simulates the behavior of the adipose tissue in the mammal suffering from hypopituitarism. The normal tadpole completing metamorphosis retains but a vestige of the former robust organ. Similarly, the fat-organ of a thyroidectomized tadpole subjected to inanition, or metamorphosed by thyroid administration, is greatly decreased in size. This is not the case with the albino (fig. 60). After prolonged starvation no decrease in the large size attained by this organ in these specimens is evident. In the 'partial' albino, too, although the animal undergoes a prolonged metamorphic period, this organ appears not to be reduced in size, though the nutritional needs of such an animal must be extreme. This was well shown by the two specimens of figure 53. The partial albino, the most emaciated of the two, had an immense fat-organ; indeed, the fat-organ and the kidneys were the major abdominal structures. This 'organ' in the other specimen, however, a normal, was scarcely recognizable. The large size and persistency of this structure in the tadpoles suffering from a complete or partial ablation of the epithelial component of the pituitary, appears not to be unlike the adiposity exhibited by the mammal after partial hypophysectomy (*adipositas universalis*, Cushing) or in the human subject suffering from hypopituitarism. These animals thus furnish abundant testimony of the disturbance in fat metabolism brought about by these conditions and indicate that even under the extreme exigencies of inanition the utilization of the fat is impossible.

5. DISCUSSION

It has been possible to determine the contribution which each group of pigment cells makes to the striking picture of 'albinism' produced by the operation of epithelial hypophysectomy in the early larvae of the toad and frog. Evidence from at least two sources (feeding experiments and epithelial exchanges) points to the importance of the paucity of the epidermal melanin in the formation of this picture. By this paucity and the consequent greater transparency of the epidermis the double sheet of broadly expanded xantholeucophores are permitted to display their full iridescent and metallic effect. The expansion of the xantholeucophore group of cells is essential to this picture, since with their contraction the albinous picture lost its characteristic metallic appearance and the larvae became notably darkened in color. Moreover, that the participation of the deep melanophores in this picture was of no great significance was evident not only from the masking which these cells suffer due to the expanded 'interference' cells, but from a variety of experimental procedures.

The atypical physiological condition of the pigment cells contributing to this picture of albinism is unquestionably referable to a fault in the endocrine system and not to an altered nervous mechanism. Not only is evidence of an unequivocal nature presented on this point as regards the altered physiological state of the xantholeucophores and epidermal melanophores by the skin exchanges, but the effect of endocrine extracts and diets on the atypical pigmentary system lends adjuvant evidence.

With the complex interrelationships obtaining in the internal secretory system, however, some difficulty has been encountered in referring the pigmentary disturbance exhibited by the tadpoles suffering from hypophysial deficiency to a definite locus in this system. Yet by the correlation of the evidence accruing both from the structural pictures presented by the members of this system together with the alterations in the pigmentary system produced by various dietary régimes and by immersion

of the albino in endocrine extracts, the specific endocrine deficiency would appear to be disclosed. In this we have been greatly aided by the different endocrine pictures obtaining in completely and partially hypophysectomized larvae, for it will be recalled that both of these types of animals exhibit a pigmentary fault. Since all the endocrine glands save the pituitary are of normal structure in the partially hypophysectomized (but albinous) larvae, it would appear that neither the adrenal components, the epithelial bodies, nor the thyroid are at fault. The thyroid can further be freed from participation in this disturbance since the pigmentary system of the thyroidectomized tadpole is normal. Further, it will be recalled that the feeding of posterior-lobe tissue (including the pars intermedia), alone, of all the administered glandular substances (thyroid, adrenal cortex, adrenal medulla, and anterior lobe), effected a partial replacement of the epidermal melanin, although, curiously, at the same time, increasing the abnormal contraction of the epidermal melanophores. It was further shown that the immersion of these larvae in pars intermedia emulsion alone of the internal secretory extracts produced a normal functional state in the chromatophore system. Thus, the evidence derived from these three lines of investigation all points to a fault in the posterior-intermediate lobe secretory mechanism as being responsible for the pigmentary disturbance obtaining in the larvae suffering from either a partial or total loss of their epithelial hypophysis.

The alimentary assimilation of the fresh glandular lobe of the pituitary has supplied the growth-maintaining principle to the animals exhibiting a retarded growth rate induced by buccal hypophysectomy.⁴⁶ Unequivocal proof of this is furnished by

⁴⁶ What element of this structurally diverse gland contributes this growth principle is obscure. Histological examination of frozen sections (15 to 20 μ) reveals that extraction with boiling absolute alcohol does not appreciably diminish either the number or size of the most characteristic element of this gland, the acidophilic granulations. Evidence determinative of the dissolving action of boiling distilled water is more uncertain because of the macerating action upon the connective tissues and the consequent difficulty in the examination of this material. Yet it is certain that a considerable number of these granules survive such treatment. That the tissue thus extracted exhibits the characteristic growth effects of the fresh gland, while the extracts do not exhibit such effects, supplies evidence against these granu-

the frog, supportive evidence by the toad tadpole. Extraction of this anterior-lobe substance with either boiling absolute alcohol or boiling water appears not to remove this principle, since the albinous tadpoles supplied with such extracts exhibit the retarded growth rate typical of the liver-fed albino, while those larvae supplied with the residues remaining after extraction grow at a normal rate.

We have pointed out the structural modifications in the various organs of the internal secretory system resulting from the early ablation of the pars epithelialis of the hypophysis. By these alterations eloquent testimony as to the interdependence of the various widely separated members of this correlative system has been secured. It will be recalled that the response of these organs to the complete loss of the epithelial hypophysis led to a diminution in their size, save in one gland, namely, the adrenal medulla. It was also pointed out that the opposite response is evoked by the early removal of the thyroids, the members of this system undergoing an increase in size, save again the adrenal medulla, which appears not to be seriously altered. It is thus seen that the response of the organs of internal secretion (save the adrenal medulla) to epithelial hypophysectomy is in the reverse direction to that resulting from thyroidectomy, the loss of the hypophysis causing a diminution, the loss of the thyroid an increase in their size.

It was further pointed out that the presence of a relatively minute remnant of the epithelial pituitary was sufficient to abort the structural anomalies in these organs arising from the complete loss of the epithelial hypophysis, save the case of the derivatives of the infundibular process. These derivatives, of which the major member is the neural lobe, are always affected in any partial epithelial hypophysectomy. This we were inclined to refer to the close anatomical relationship obtaining between these two components of the pituitary. For it was shown that

lations being merely a by-product or a refuse accumulated during the secretory process. It must be admitted that such evidence is inconclusive, however, since the part played by the other cells, the basophiles and chromophobes, whose cytoplasm appears to be more labile than that of the acidophiles, has not been excluded.

in the case of the complete absence of the epithelial hypophysis the neural lobe underwent an abortive development, while the other portion of the infundibular process, the pituitary floor, normally in contact with the epithelial component, retained its membranous character in sharp contrast to the thickened wall normally displayed. Further studies of the 'partial' albino indicated that the atypically placed epithelial fragment apparently is able to 'stimulate' the adjacent neural tissue to form a structure, simulating histologically the typical neural lobe. Doubt, however, was cast upon the functional sufficiency of this 'novel' neural lobe, since the animals displaying this anatomical arrangement failed to metamorphose in contrast to those in which this epithelial fragment attained contact, though but slight, with the true neural lobe, a failure not entirely attributable to the smaller size of this epithelial remnant. From this it would appear that the neural tissue comprising the pars nervosa of the pituitary enjoys the same functional specificity as do the other glandular tissues of the body.

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6. SUMMARY

1. The frog and the toad tadpole, because of the accessibility of the epithelial hypophysis in the early larval stages and the prolonged survival of the animals suffering the loss of this gland, has proved of great value in the institution of hypophysial disturbances and in the subsequent analysis thereof.

2. The early removal of the epithelial hypophysis induces a striking disturbance, 1) in the pigmentary system, 2) in the growth rate, and, 3) in the structural characteristics of most of the other glands of internal secretion.

3. The pigmentary alterations resulting from early hypophysectomy are expressed by, 1) a diminution in the epidermal free pigment; 2) a diminution in the number and melanin content of the epidermal melanophores, together with an abnormal state of contraction; 3) a maximal expansion of the xantholeucophores; 4) a partial contraction of the deep melanophores in the younger larvae.

4. The first three above-mentioned alterations in this system result in and are essential to the complete formation of the picture of albinism; the fourth does not materially contribute to this picture. This would be suspected from the anatomical arrangement obtaining in these cell layers and can further be proved by various experimental procedures by which each of these factors can be modified: 1) the partial replacement of the epidermal melanin (effected by posterior-lobe feeding) is seen to blanket partially the underlying, broadly expanded xantholeucophores, the larvae being notably darker than their brothers supplied with other diets; 2) the complete expansion or contraction of the deep melanophores of the albino effected by altering the conditions of light and background does not materially change the color of the albinous larvae, and conversely the contraction of these cells in the normal by the absence of light, while productive of a translucency, does not produce an albino; 3) a contraction of the xantholeucophores of the albino as exhibited by an albinous graft to a normal host or by the use of a strong anaesthetic not only results in their darkening, but in the loss of the metallic silvery tone characteristic of the picture of albinism.

5. A developmental study of the pigmentary system of the albino reveals the fact that the epidermal melanophores appear at a later date and in diminished numbers as compared to the normal; that the free epidermal melanin suffers a relatively greater diminution than in the normal, and that the xantho-

leucophores appear at the same time and in approximately the same numbers as in the normal, but exhibit from their earliest appearance a broad expansion as compared to the punctate character of these cells in the unoperated tadpole.

6. The broad expansion of the xantholeucophores and contraction of the epidermal melanophores of the albino is not due to an alteration in their nervous mechanism, but to the modified tissue fluids which bathe them. This is proved by the reciprocal skin exchanges. That this alteration in the tissue fluids is of a hormonal nature appears probable not only from the known potency of these substances, but also because of the extensive modifications suffered by the elaborators of these substances in the albino. There appears to be no ground for referring the deficiency in the epidermal melanin to other than an internal secretory origin.

7. Various physiological and pharmacological experiments on the pigmentary system of older albinous and normal larvae reveal the fact that, 1) the deep melanophores of the albino and the normal tadpole react identically under all tests tried by the author; 2) the xantholeucophores of the normal animal respond to changes in environmental conditions, those of the albino, on the other hand, maintain a refractory expansion under all sublethal stimuli tried by the author, save that afforded by the pars intermedia emulsion; 3) the epidermal melanophores of the albino invariably expand when subjected to the condition of 'light and heat.' Those of the normal tadpole react in a more variable manner, not infrequently exhibiting the opposite reaction, contracting instead of expanding.

8. The thyroid and the adrenal cortex are strikingly diminished in size in the albino; the epithelial bodies suffer a lesser though definite diminution; the adrenal medulla suffers no unquestionable quantitative changes, but it appears not improbable that it is slightly hypertrophied. Structurally, the adrenal medulla is clearly altered in the albino, since its cells are all of a uniformly staining large type, as compared to the variability in size and the diversity in appearance exhibited by the adrenal chromaffin cells of the normal.

9. The posterior lobe is invariably diminished in size, asymmetrical in shape, and atypical in position in the albino. Moreover, in the albino the floor of the infundibular process—pituitary floor—normally in contact with the epithelial hypophysis, does not undergo the secondary thickening which takes place in the normal subsequent to the juxtaposition of the epithelial hypophysis with this structure, but retains its membranous structure.

10. Attempts to remove the buccal hypophysis occasionally leave a sufficiently large fragment of the epithelial hypophysis in place for complete regeneration to occur. Other instances of incomplete removal of the epithelial part of the gland produce animals which are altered in a characteristic way. We have termed these 'partial' albinos. As far as their appearance is concerned, in most instances these animals are typical albinos and the incomplete nature of the operation is only disclosed later when a development of the limbs appears. Indeed, some of these 'partial' albinos furnished the only instances of the complete metamorphosis of albinous larvae. Our present knowledge of the intimate relationship of the thyroid to metamorphosis would lead us to predict that the thyroid gland at least, was not interfered with. As a matter of fact, anatomical study of all of the 'partial' albinos showed that not only the thyroid, but that all of the internal secretory glands, with one exception, were undiminished in size or degree of development. Indeed, in a limited number of those 'partial' albinos which completed metamorphosis the thyroid was greatly hypertrophied (colloid goiter) and frequently accessory thyroids were formed.

11. The neural lobe of the 'partial' albino is usually diminished in size and is invariably atypical in shape and position.

12. About the atypically placed epithelial fragment of the hypophysis of the 'partial' albino there is formed a 'novel' neural lobe, a structure apparently formed not only by the indentation of this epithelial fragment in the brain tissue, but by an actual hypertrophy of the adjacent parts. Not infrequently in these specimens there is no recognizable neural lobe such as invariably is exhibited by the typical albino.

13. The evidence thus indicates that the neural lobe and pituitary floor are dependent upon the epithelial hypophysis for their complete development, and further that an atypically placed epithelial hypophysis has the power to cause an hypertrophy of the adjacent neural tissue.

14. Since 'partial' albinos may be secured in which the pigmentary upset or albinism is nearly as profound as in those larvae which have suffered a complete epithelial ablation, and since in these instances none of the endocrine glands are gravely impaired in development save the hypophysis, and since the feeding of posterior lobe (plus pars intermedia), alone, of the major internal secretory glands effects a partial replacement of the epidermal melanin in the albino, and further since no extract save that of the pars intermedia induces a normal physiological state in the pigmentary system of this animal, it seems justifiable to refer the endocrine fault responsible for the pigmentary disturbance to the posterior lobe or the posterior-intermediate lobe secretory mechanism and to free the other glands from responsibility.

15. The frog tadpole suffering from complete or incomplete hypophysial deficiency exhibits even after a long period of inanition or after a prolonged metamorphosis a fat-organ undiminished in size, in contrast to the small size of this organ in similarly treated thyroidless tadpoles or in normal tadpoles near the end of metamorphosis. The persistence of the fat-organ in these tadpoles appears to simulate the adiposity exhibited by the mammal suffering from hypophysial deficiency.

16. The frog tadpole which has suffered the early loss of its epithelial hypophysis exhibits a retardation in growth when supplied with a continuous diet of liver, posterior lobe, adrenal cortex, or adrenal medulla. This growth retardation is feebly expressed at first, but becomes very pronounced at about the midlarval period.

17. A continuous diet of the fresh anterior lobe of the beef replaces the growth-'maintaining' substance lost by buccal hypophysectomy and so effects a nearly normal rate of growth in the albinous frog tadpole. Since these animals do not meta-

morphose, their growth extends beyond the normal larval period. Consequently they attain a size in excess of the normal.

18. The sensitiveness of the hypophysectomized frog tadpole to the growth-‘maintaining’ substance of the anterior lobe makes it possible by feeding experiments to test in a particularly efficacious manner any hypophysial substance for the presence or absence of this principle.

20. Neither aqueous nor alcoholic extracts of the anterior lobe of the pituitary nor the intraglandular colloid of the hypophysis contain the growth-‘maintaining’ substance of this gland. Conversely, the residues formed by extracting the hypophysis with boiling water or boiling absolute alcohol contain this growth substance.

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PLATES

ABBREVIATIONS

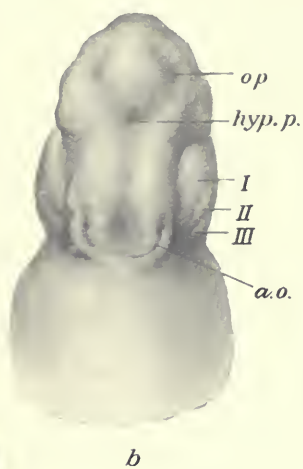
<i>a.o.</i> , adhesive organ	<i>op.v.</i> , optic vesicle
<i>hyp.</i> , hypophysis	<i>ot.v.</i> , otic vesicle
<i>hyp.p.</i> , hypophysial pit	<i>p.f.</i> , pituitary floor
<i>inf.</i> , infundibulum	<i>p.g.</i> , pars glandularis of the hypophysis
<i>n.p.ep.</i> , vestigial epithelial lobe (pars epithelialis)	<i>p.i.</i> , pars intermedia of the hypophysis
<i>n.p.n.</i> , new neural lobe	<i>p.n.</i> , pars neuralis of the hypophysis
<i>o.p.</i> , olfactory placode	<i>t.p.f.</i> , thickened portion of pituitary floor
	<i>I, II, III</i> , branchial arches

PLATE 1

EXPLANATION OF FIGURES

11 A 4-mm. frog larva (*R. boylei*) showing the surface characteristics at a favorable stage for epithelial hypophysectomy. (a) Ventrolateral, (b) ventrocephalic view.

12 A median sagittal section of a frog larva of approximately the same age and size as shown in figure 11.



11

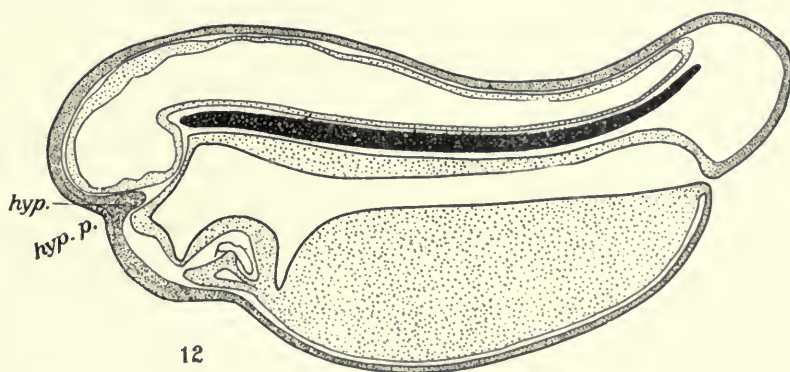


PLATE 2

EXPLANATION OF FIGURES

13 The pigmentary system⁴⁷ of a normal 43/9.0 ⁴⁸frog tadpole, 'standard' environment, showing the successive layers of free pigment and chromatophores.⁴⁹ The animal was fixed by dropping into Helly's fluid 101 days after the operative stage. Liver diet. $\times 226$.

14 The pigmentary system of a 43/0.1 albino frog tadpole, 'standard' environment. Fixed by dropping into Helly's fluid 101 days after epithelial hypophysectomy. Liver diet. $\times 226$.

15 The pigmentary system of a 10-mm. (total length) normal frog tadpole, 'standard' environment. Dropped into Helly's fluid 22 days after the operative stage. Liver diet. The epidermal melanophores are just appearing. $\times 226$.

⁴⁷ All the drawings of the pigment cells (figs. 13 to 23) were made from skin whole mounts taken from the dorsal region of the body.

⁴⁸ The total length of the specimen is shown by the first figure, the hind-leg length by the second.

⁴⁹ In the pigment cell drawings the most superficial layer is shown on the left, the deepest on the right. From left to right they are in order, (a) the layer of free epidermal pigment, (b) the layer of epidermal melanophores, (c) the xantholeucophores, (d) the deep melanophores.

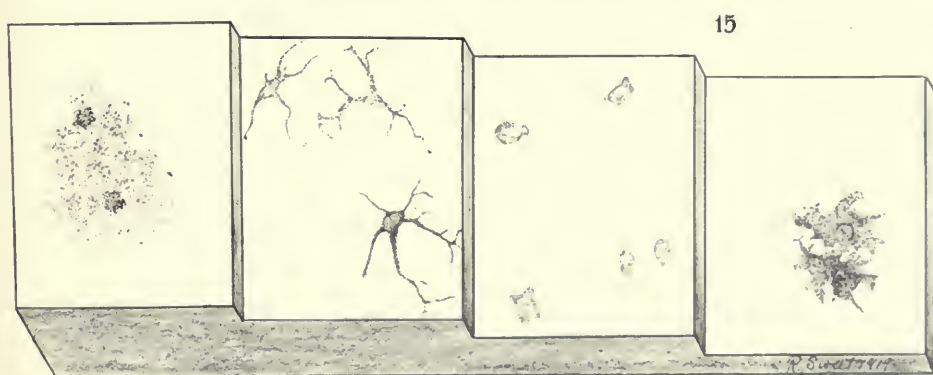
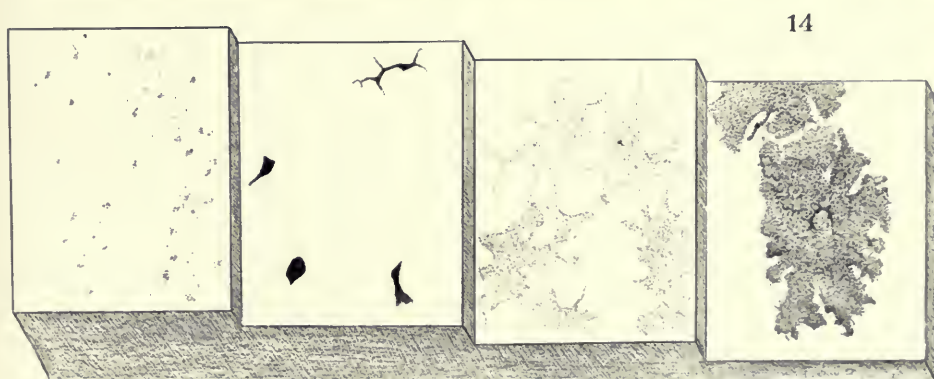
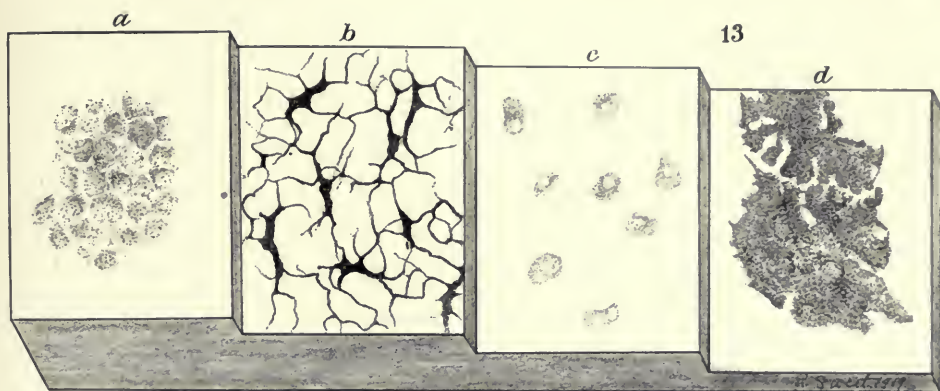


PLATE 3

EXPLANATION OF FIGURES

16 The pigmentary system of a 10-mm. (total length) albino frog tadpole, 'standard' environment. Dropped into Helly's fluid 22 days after the operative stage. Liver diet. The epidermal melanophores have not yet formed. $\times 226$.

17 The pigmentary system of a 14.5-mm. (total length) normal frog tadpole, 'standard' environment. Fixed by dropping into Helly's fluid 47 days after the operative stage. Liver diet. $\times 226$.

18 The pigmentary system of a 14.5-mm. (total length) albino frog tadpole, 'standard' environment. Fixed by dropping into Helly's fluid 47 days after the operative stage. Liver diet. $\times 226$.

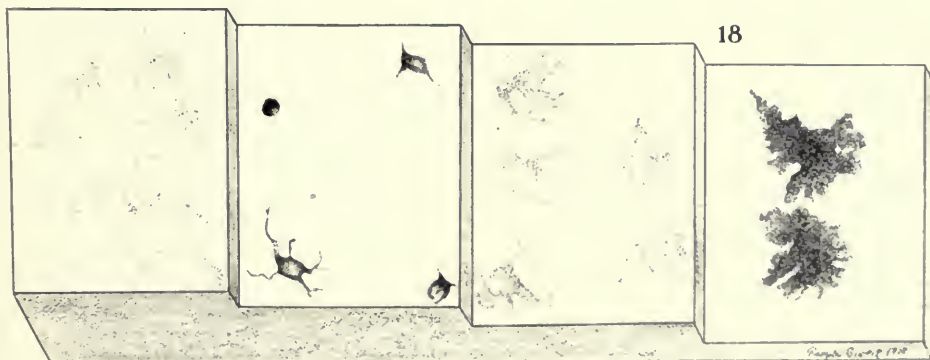
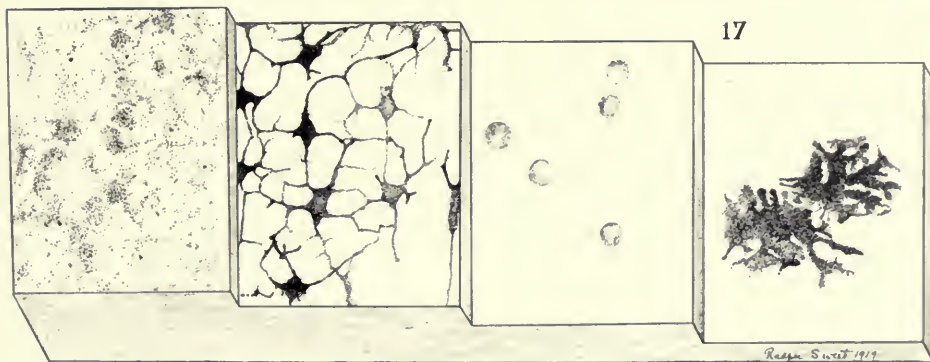
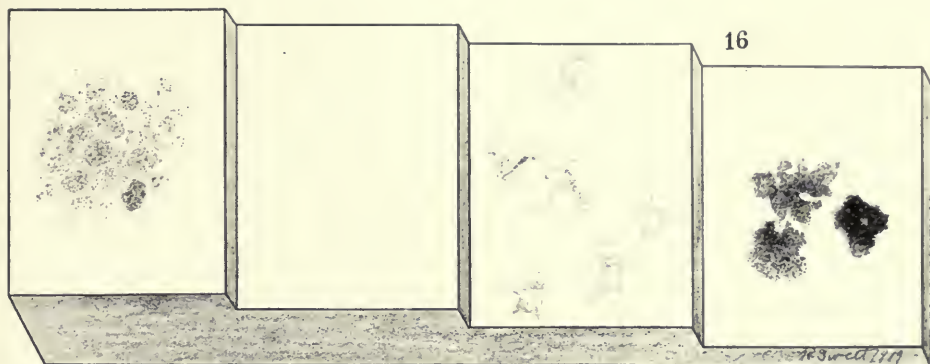


PLATE 4

EXPLANATION OF FIGURES

19 The pigmentary system of a 'light and heat' adapted 38/6.0 normal frog tadpole which exhibited a contraction of the epidermal melanophores under the influence of this stimulus. Fixed $5\frac{1}{2}$ months after the operative stage by dropping into Helly's fluid. Liver diet. $\times 226$.

20 The pigmentary system of a 'light and heat' adapted 46/0.5 albino frog tadpole. Fixed $5\frac{1}{2}$ months after epithelial hypophysectomy by dropping into Helly's fluid. Liver diet. $\times 226$.

21 The pigmentary system of a 46/0.1 albino frog tadpole, supplied with a continuous diet of posterior lobe, 'standard' environment. Fixed by dropping into Helly's fluid, $4\frac{1}{2}$ months after epithelial hypophysectomy. $\times 226$.

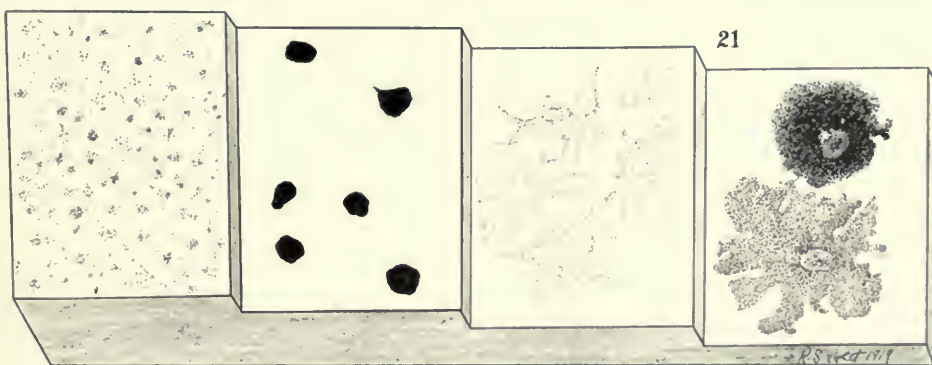
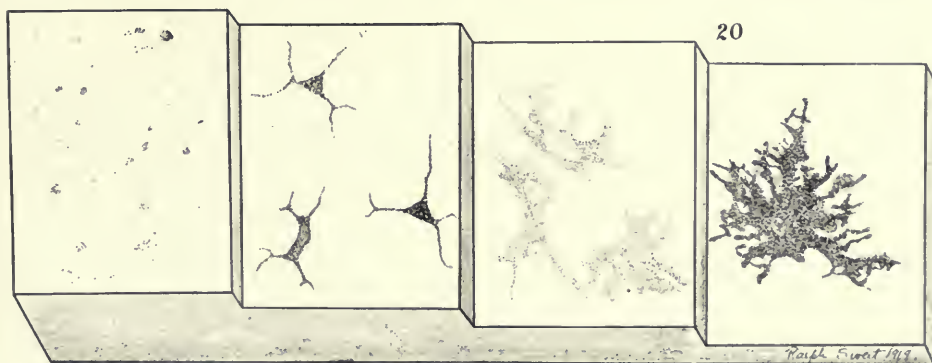
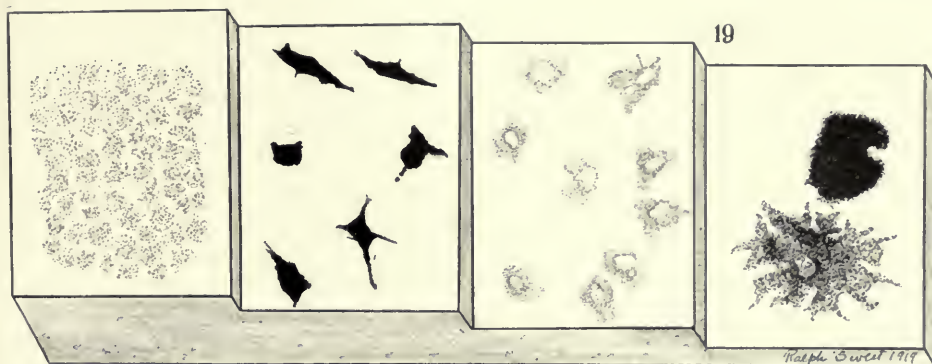


PLATE 5

EXPLANATION OF FIGURES

22 The pigmentary system of a 46/0.1 albino frog tadpole, 'light and heat' adapted. This tadpole was supplied with a posterior-lobe diet for $4\frac{1}{4}$ months. Prior to fixation it had been on a liver diet for one week. Fixed $4\frac{1}{2}$ months after epithelial hypophysectomy. $\times 226$.

23 The pigmentary system of a 43/0.1 albino frog tadpole, 'light and heat' adapted. This tadpole was supplied with a posterior-lobe diet for $3\frac{1}{4}$ months. It had been on a liver diet for 5 weeks prior to fixation. Fixed by dropping into Helly's fluid $4\frac{1}{2}$ months after epithelial hypophysectomy. $\times 226$.

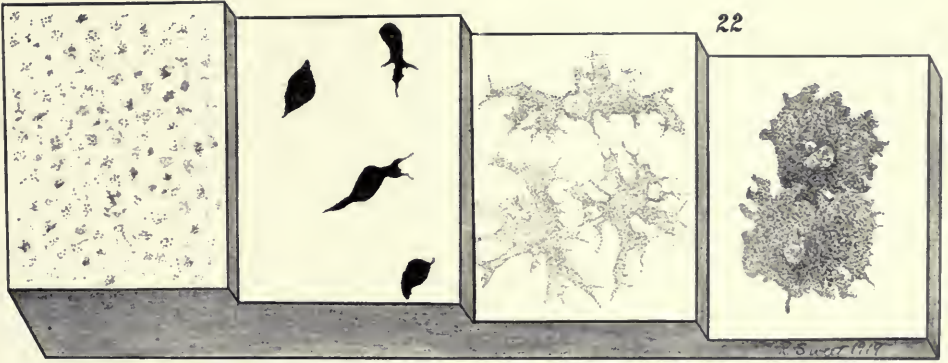


PLATE 6

EXPLANATION OF FIGURES

24 A diagram of the ventral view of the brain of a 45/6.0 frog tadpole to show the position of the hypophysis.

25 A model of the infundibular process and hypophysial components of a 38/3.5 normal frog tadpole (specimen 15), fixed 63 days after the operative stage. Liver diet. The caudal end of the infundibular process faces the top of the page. *a*, dorsal, *b*, ventral, *c*, median sagittal, views. $\times 89$.

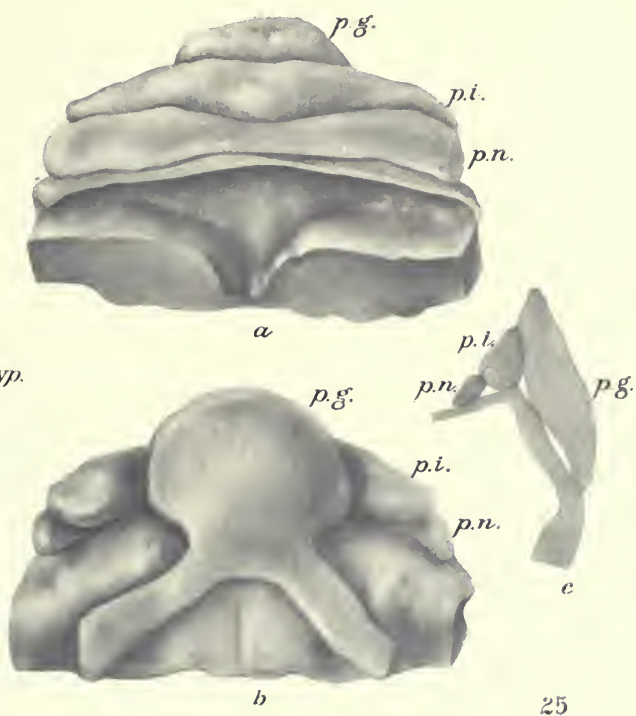
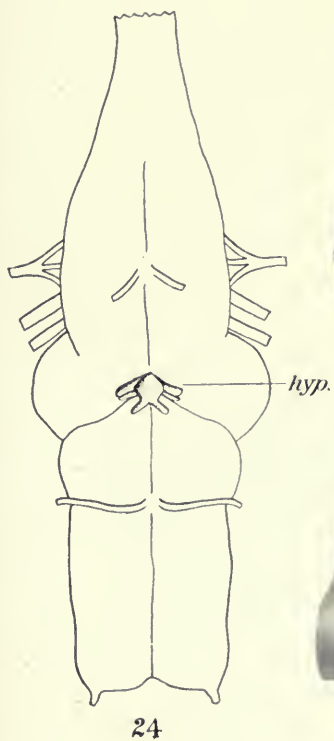
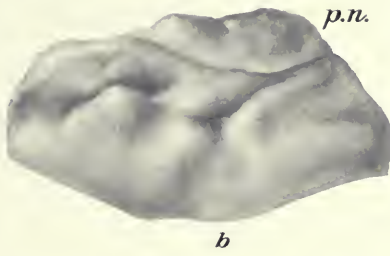
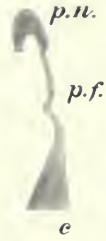
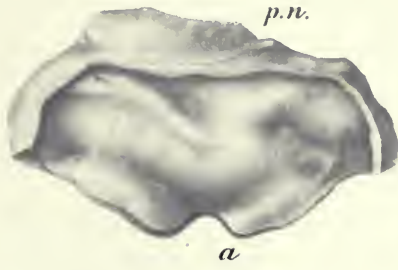


PLATE 7

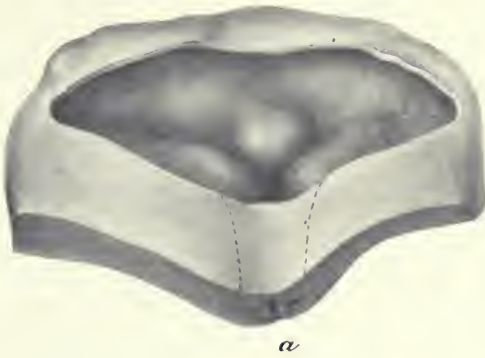
EXPLANATION OF FIGURES

26 A model of the infundibular process and hypophysial components of a 43/0.1 albino frog tadpole (specimen 18), fixed 101 days after epithelial hypophysectomy. Liver diet. The caudal end of the infundibular process faces the top of the page. *a*, dorsal, *b*, ventral, *c*, median sagittal, views. $\times 89$.

27 A model of the infundibular process and hypophysial components of a 36/4.2 'partial' albino frog tadpole (specimen, p 1), fixed 66 days after the operative stage. Liver diet. The caudal end of the infundibular process faces the top of the page. *a*, dorsal, *b*, ventral, *c*, median sagittal, views. $\times 89$.



26



27

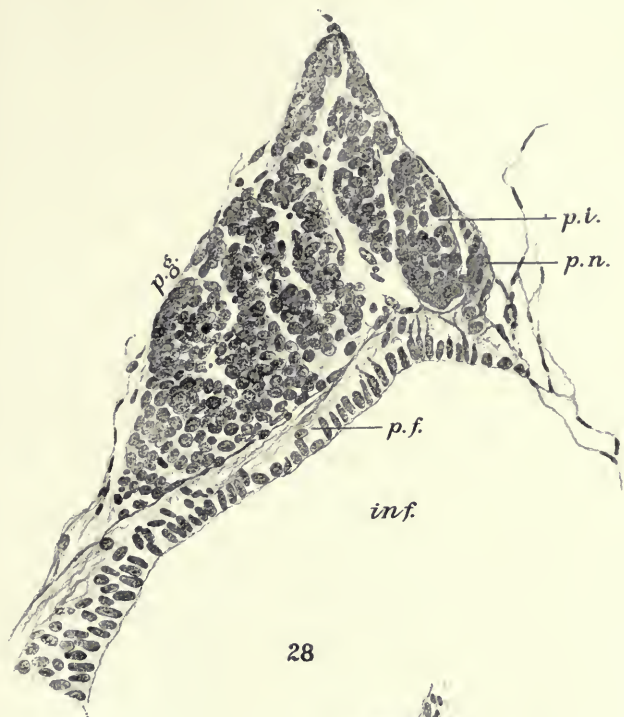
PLATE 8

EXPLANATION OF FIGURES

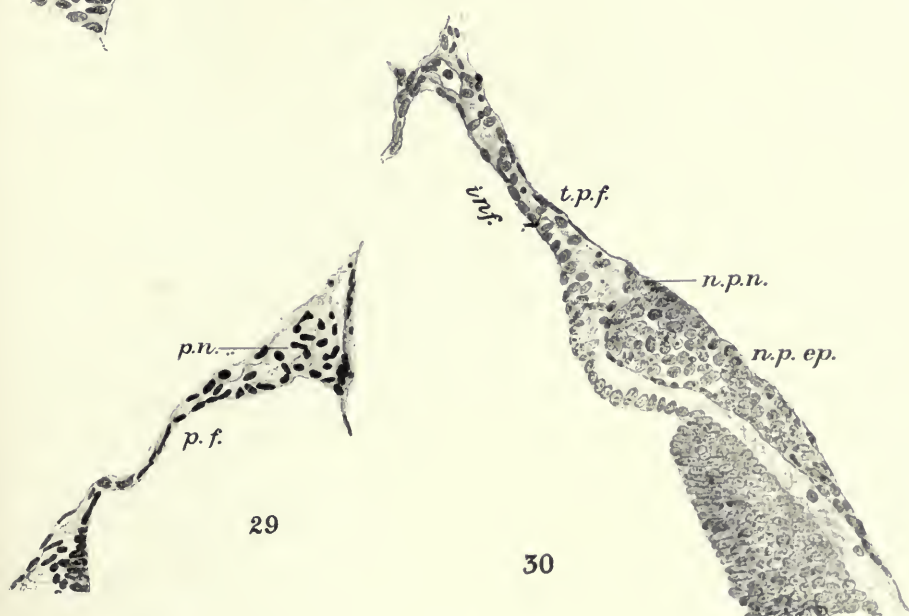
28 A median sagittal section through the infundibular process and the hypophysial components of a 38/3.5 normal frog tadpole. Liver diet. Fixed 63 days after the operative stage. $\times 227$.

29 A median sagittal section through the infundibular process and the hypophysial components of a 43/0.1 albino frog tadpole (specimen 16). Liver diet. Fixed 101 days after epithelial hypophysectomy. $\times 227$.

30 A median sagittal section through the infundibular process and the hypophysial components of a 36/4.2 'partial' albino frog tadpole (specimen p 1). Liver diet. Fixed 66 days after the attempted epithelial hypophysectomy. $\times 227$.



28



29

30

PLATE 9

EXPLANATION OF FIGURES

31 (a) Ventral and (b) median views of a model of the left thyroid of a 38/3.5 normal frog tadpole (specimen 15). Liver diet. Fixed 63 days after the operative stage. $\times 89$.

32 (a) Ventral and (b) median views of a model of the left thyroid of a 43/0.1 albino frog tadpole (specimen 18). Liver diet. Fixed 101 days after epithelial hypophysectomy. $\times 89$.



PLATE 10

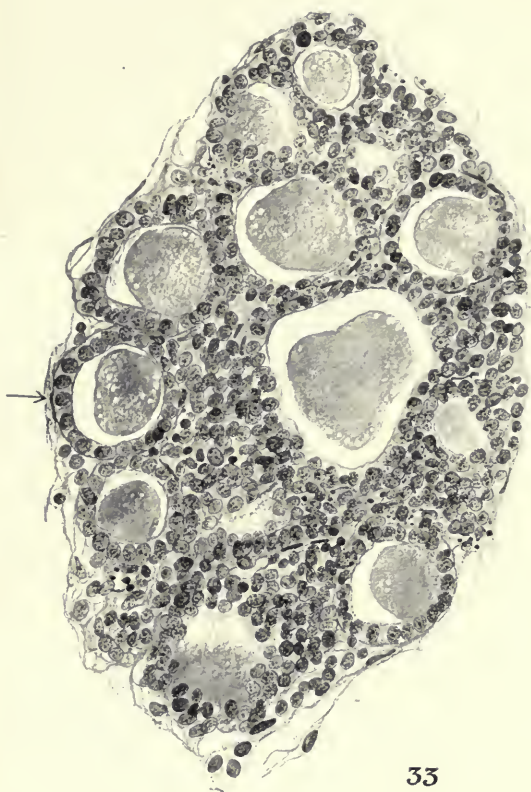
EXPLANATION OF FIGURES

33 A cross-section through the largest portion of the left thyroid of a 40/5.0 normal frog tadpole. Liver diet. Fixed 64 days after the operative stage. $\times 227$.

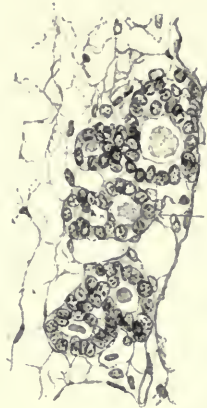
34 A cross-section through the largest portion of the left thyroid of a 40/1.5 albino frog tadpole. Liver diet. Fixed 64 days after epithelial hypophysectomy. $\times 227$.

35 The follicle, shown by an arrow, in figure 33. $\times 765$.

36 The follicle, shown by an arrow, in figure 34. $\times 765$.



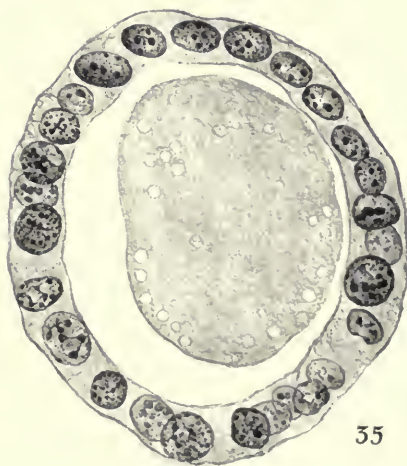
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34



36



35

PLATE 11

EXPLANATION OF FIGURES

37 The ventral surface of the dorsal abdominal wall of a 44/9.0 frog tadpole to show the position of the adrenals.

38 The adrenal cortex of a 43/8.0 normal frog tadpole. Drawn from a whole mount. Osmium-bicromate fixation. The specimen was killed 221 days after the operative stage. Liver diet. $\times 24$.

39 The adrenal cortex of a 55/0.2 albinous frog tadpole. Same fixation, age, and diet as above. $\times 24$.

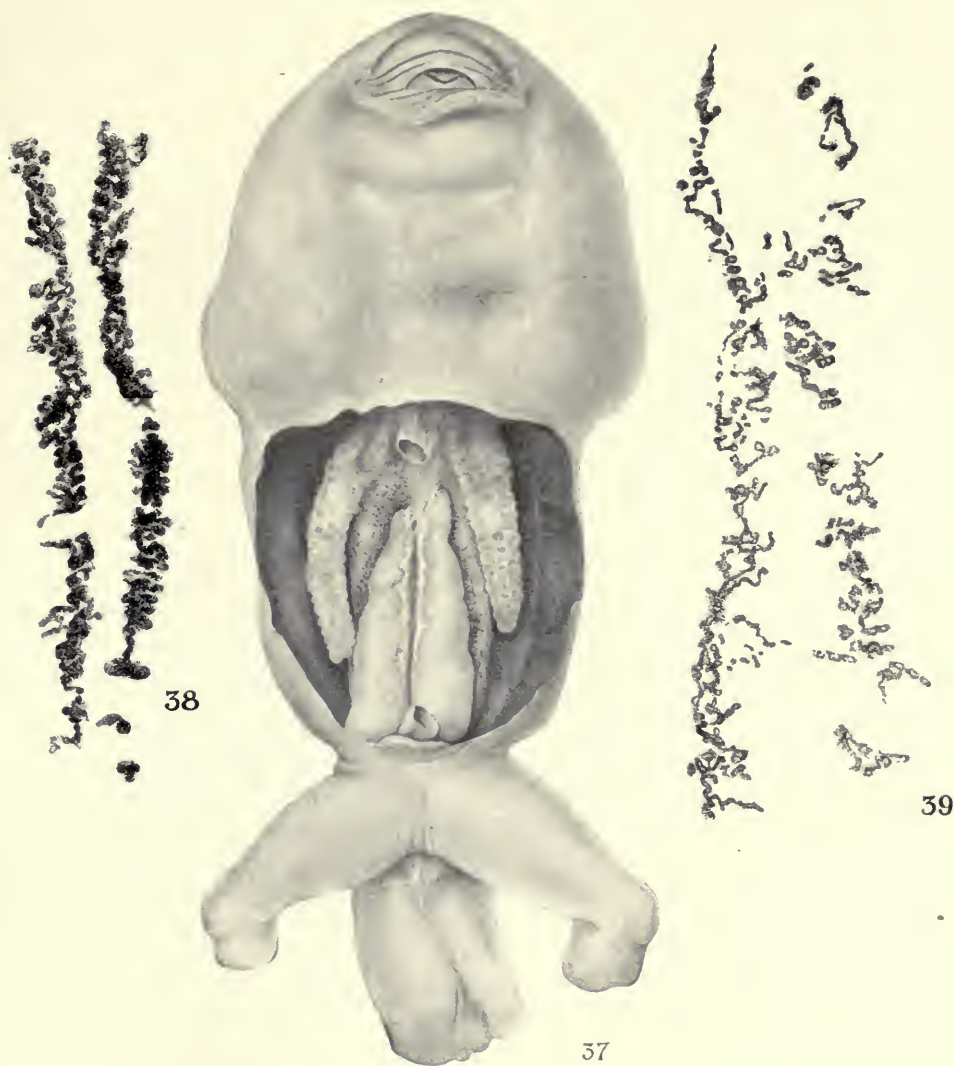
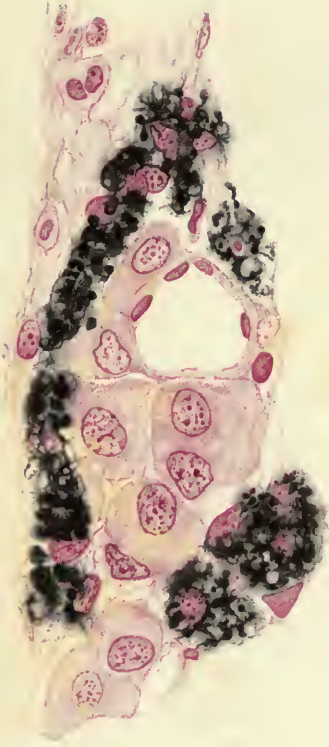


PLATE 12

EXPLANATION OF FIGURES

40 A small portion of the adrenal of a 48/9.0 normal frog tadpole. Liver diet. Fixed in osmium-bichromate 195 days after the operative stage. Babes' safranin stain. Cortex black, medulla and nuclei red. $\times 733$.

41 A small portion of the adrenal of a 54/0.2 albino frog tadpole. Same age, diet, fixation, and stain as above. $\times 733$.



40



41

PLATE 13

EXPLANATION OF FIGURES

42. Normal and hypophysectomized frog tadpoles showing the first appearance of albinism (May 14th).⁵⁰ Age 22 days, dated from the operative stage. Liver diet. The albino is at the left. $\times 2\frac{1}{2}$.

43. The same specimens as shown in figure 42, eight days later (May 22nd). Age 30 days, dated from the operative stage. Liver diet. $\times 2\frac{1}{2}$.

44. The same specimens as shown in figure 42, twelve days later (May 26th). Age 34 days, dated from the operative stage. Liver diet. $\times 2\frac{1}{2}$.

45. A pair of normal and albinous frog larvae photographed June 12th. Age 50 days, dated from the operative stage. Liver diet. $\times 2\frac{1}{2}$.

⁵⁰ All the photographs presented in the paper were taken by our scientific illustrator, Mr. Matthews. I wish to emphasize my great appreciation for the untiring effort and skillful work of Mr. Matthews which has enabled me to present with great fidelity the appearance of these specimens.



45



44



43



42



PLATE 14

EXPLANATION OF FIGURES

46. A normal and an albinous frog tadpole photographed July 10th. Liver diet. Age 78 days, dated from the operative stage. $\times 2\frac{1}{2}$.
47. A normal and an albinous frog tadpole of the same age and photographed on the same date as those shown in figure 46, showing the effect of a posterior-lobe diet upon albinism. Age 78 days, dated from the operative stage. $\times 2\frac{1}{2}$.
48. A normal and an albinous frog tadpole of the same age and photographed on the same date as those shown in figures 46 and 47, supplied with anterior-lobe substance. Age 78 days, dated from the operative stage. $\times 2\frac{1}{2}$.



46



47



48



PLATE 15

EXPLANATION OF FIGURES

49. A normal and an albinous frog tadpole photographed on August 9th. Liver diet. Age 108 days, dated from the operative stage. $\times 2\frac{1}{2}$.
50. A normal and an albinous frog tadpole of the same age and photographed on the same date as the specimens shown in figure 49. Posterior-lobe diet. Age 108 days. $\times 2\frac{1}{2}$.
51. A normal and an albinous frog tadpole of the same age and photographed on the same date as the specimens shown in figures 49 and 50. Anterior-lobe diet. Age 108 days. $\times 2\frac{1}{2}$.



49



50



51

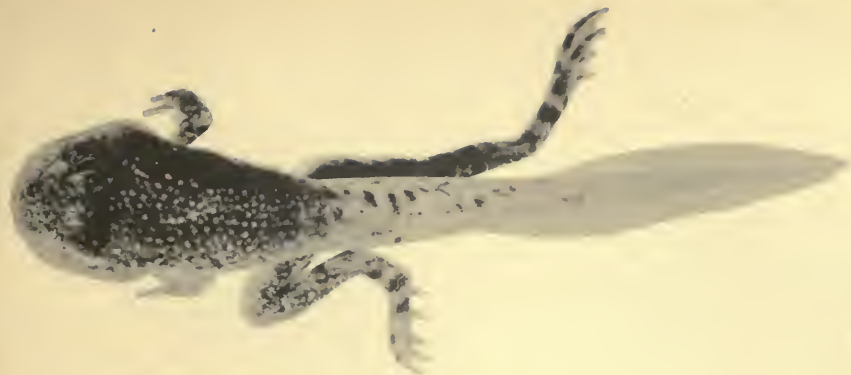


PLATE 16

EXPLANATION OF FIGURES

52. A frog 'partial' albino (specimen p. 4) photographed on October 9th, and a liver-fed normal photographed on September 5th. Ages 221 and 185 days respectively, dated from the operative stage. $\times 2$.

53. (a) A frog 'partial' albino (specimen p. 7) which completed metamorphosis, (b) A normal frog (specimen 33) immediately after metamorphosis. The metamorphosis of both of these specimens was delayed. Photographed on April 7, 1919. Age 356 days. $\times 2$.

54. A toad 'partial' albino (specimen p. 10) and a normal toad. Photographed May 1, 1919. The albino completed metamorphosis on November 25th. The time of metamorphosis of the normal is unknown. Age 335 days. $\times 2$.

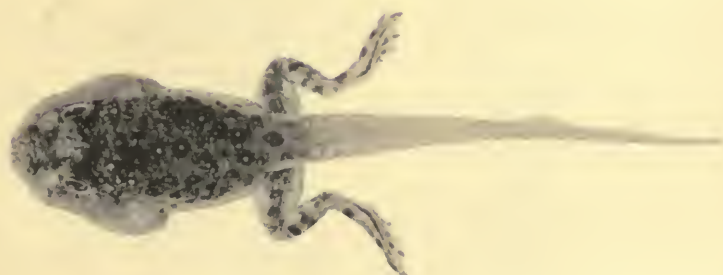


a

53



b

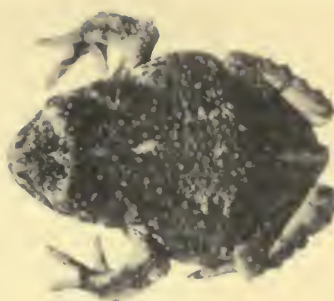


b

52

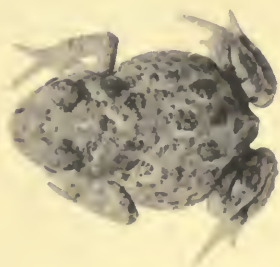


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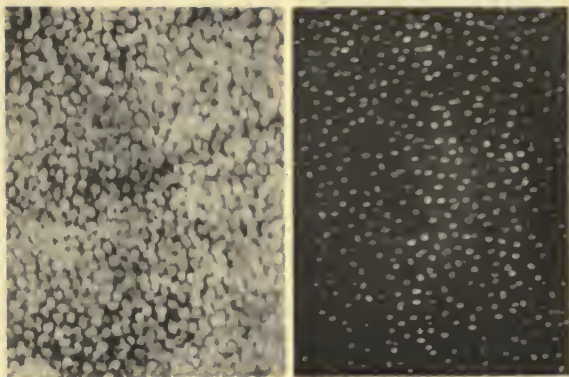
a

PLATE 17

EXPLANATION OF FIGURES

55. Photographs of a normal and an albinous frog tadpole to show the xantho-leucophores. Photographed May 26th. The same specimens as shown in figure 44. $\times 7$.

56. An enlargement of a portion of the dorsal body surface of the specimens shown in figure 55.



56



55

PLATE 18

EXPLANATION OF FIGURES

57. An albinous and a normal frog tadpole showing reciprocal skin exchanges. Taken four hours after the skin exchange was effected. Age of specimens 70 days, dated from the operative stage. $\times 5$.

58. Enlargements of the skin exchanges and surrounding region of the specimens shown in figure 57.



58



57

PLATE 19

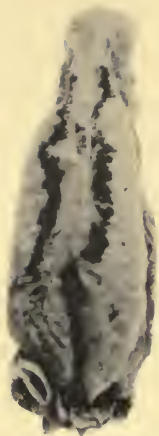
EXPLANATION OF FIGURES

59. The mesonephroi and adrenal bodies, fixed in the osmium-bichromate solution. (a) 'Partial' albino young adult frog (specimen p. 7). (b) Normal young adult frog (specimen 33). (c) Albinous frog larva. Age of specimens 356 days. Specimens a and b are shown in figure 53.

60. An albinous (a) and a thyroidless (b) frog tadpole photographed to show the fat-bodies. Taken immediately after death. These specimens had been subjected to inanition for six weeks prior to death. Age 335 days. $\times 2$.



a



b



c

59



60

AMERICAN ANATOMICAL MEMOIRS

No. 1. The Anatomy and Development of the Systemic Lymphatic Vessels in the Domestic Cat, by George S. Huntington, Professor of Anatomy, Columbia University, New York City, states the various theories held in regard to lymphatic development in general and then presents the result of six years' careful investigation on mammalian lymphatic development. Part I deals with the development of the systemic lymphatic vessels in their relation to the blood vascular system. Part II deals with the development of the preazygos and azygos segments of the thoracic duct. 175 pages of text, 8 text figures (two in color), 254 photomicrographs and 21 colored plates. Sent post paid to any country for \$4.00. 1911.

No. 2. Contribution to the Study of the Hypophysis Cerebri with Especial Reference to its Comparative Histology, by Frederick Tilney, Associate in Anatomy, Columbia University, New York City. Part I contains a historical review of the literature. Part II deals with the comparative histology of the pituitary gland and gives a report of six hypophysectomies performed upon cats. 72 pages of text, 2 text figures, 60 photomicrographs and plates. Sent post paid to any country for \$1.50. 1911.

No. 3. Early Stages of Vasculogenesis in the Cat (*Felis Domestica*) with Especial Reference to the Mesenchymal Origin of Endothelium, by H. Von Schulte, Department of Anatomy, Columbia University, New York City. 90 pages of text and 33 figures, of which 14 are in colors. Sent post paid to any country for \$1.50. 1914.

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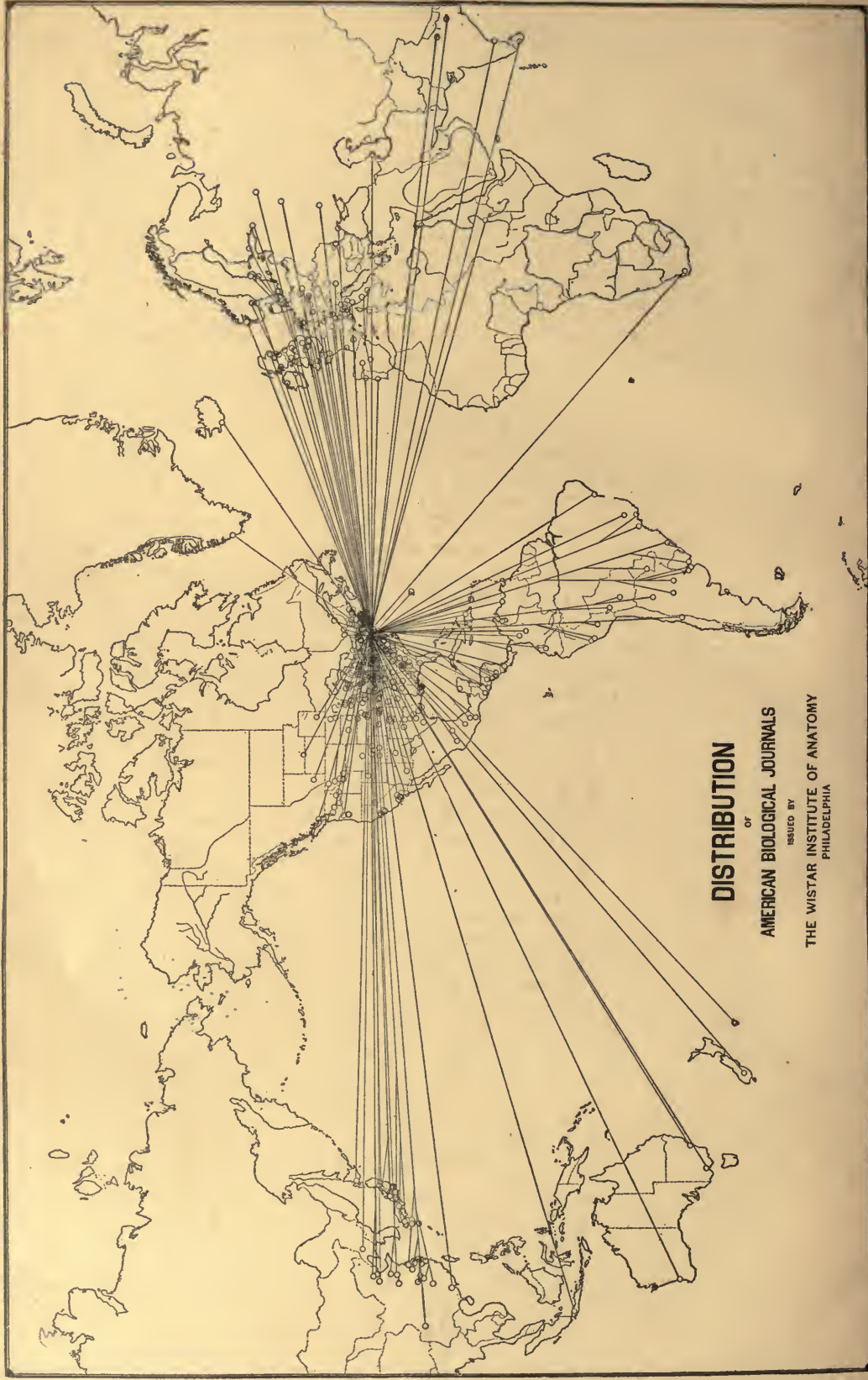
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